STUDIES OF THE TOXICITY AND PHYTOTOXICITY OF TWO SOIL APPLIED GRANULATED SYTEMIC INSECTIDES

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I N T R O D U C T I O N

Control of aphids on agricultural crops can be obtained by various cultural practices, but insecticides figure prominently in most programmes aimed at obtaining economic control of aphid species which are persistent virus vectors. A recent advance in insecticide formulations has been the development of granulated systemic aphicides.

The aim of this thesis was to investigate the properties of two of these granulated insecticides, namely Isolan, a carbamate and phorate, an organophosphate.

In particular an attempt was made to assess the persistence and phytotoxicity of these granulated systemic insecticides following application to the soil. Because Isolan and phorate are comparatively new insecticides in New Zealand, an endeavour was made to establish the relative aphicidal properties of these chemicals in the vapour phase and as contact insecticides, in order to envisage their suitability as granular applied aphicides.

C O N T E N T S

PLATES and FIGURES

NOMENCLATURE OF CHEMICALS REFERRED TO IN THIS THESIS

The names marked by 'a' are approved by the Committee of the Entomological Society of America, names marked by 'b' are adopted in the British Standard (with its supplements), those marked by 'c' have been adopted by the U.S. Interdepartmental Committee on Pest Control, and those marked by 'd' are cited in the list in Phytopathology.

COMMON NAMES	CHEMICAL NAMES OR DEFINITIONS	OTHER NAMES
aldrin [a,b,c,]	not less than 94 % HHDN (q.v.) HHDN = 1,2,3,4,10,10-hexacloro-1,4, 4a,5,8,8a,-hexa-hydro-exo-1,4,-endo- 5,8-dimethanon aphthalene, according to the nomenclature of Chemical Society London. 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a hexahydro-endo-1,4,-exo-5,8-dimethanon- aphthalene, according to the nomenclature of the American Chemical Society.	
BHC [a,b,]	1,2,3,4,5,6-hexachlorocyclohexane benezene hexachloride (BHC is used for a mixture of isomers αBHC, βBHC and γBHC. The British Standard requires that the % of γBHC be stated).	
dalapon	2,2-dichloropropionic acid	
DDT [a,b,]	a complex chemical mixture in which p,p, DDT (q.u.) predominates. (The British Standard requires the % of p,p, DDT to be stated.)	
p,p, DDT [b]	1,1-di(p-chlorophenyl)-2,2,2-trichloroet- hane	
demeton [a,b,]	a mixture of demeton-O and demeton-S (q.v.)	Systox
demeton-O [b]	O,O-diethyl O-2-(ethylthio-ethyl phosphorothioate	(Systox (thiono isomer
demeton-S [b]	O,O-diethyl S-2-(ethylthio-ethyl phosphorothioate	(Systox (thiol isomer
demeton-methyl	a 70 : 30 mexture of demeton-O-methyl and demeton-S-methyl	Metasystox

COMMON NAMES	CHEMICAL NAMES OR DEFINITIONS	OTHER NAMES
diazinon[b]	0,0-diethyl 0-2-isopropyl-4-methyl-6-pyrimidinyl phophorothioate	Diazinon[a]
dieldrin [a,b,c,]	a product containing 85 % HEOD	
dimefox [a,b,]	bis(dimethylamino)fluorophosphine oxide	Hanane, Pestox 14
dimethoate	0,0-dimethyl S-methylcarbamoyl- methyl phosphorodithioate	Rogor
Disyston	diethyl S-(2-(ethylthio)ethyl) phosphorothiolothionate	Disulfoton
HEPP	hexaethyl pyrophosphate	
HEOD	1,2,3,4,10,10-hexachlor-6,7-epoxy-1,4,4a 5,6,7,8,8a,-octahydro-exo-1,4-endo-5,8, dimethanonaphthalene	
heptachlor	1,4,5,6,7,10,10-heptachloro-4,7,8,9-tetrahydro-4,7-methyleneindene	
Isolan[a]	1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate	
lindane [a,c,]	isomer of BHC (q.v.) of not less than 99 per cent purity	
malathion [a,b,c,]	0,0-dimethyl S-(1,2-di(ethoxy-carbonyl) ethyl) phosphorodithioate	
mipafox [b]	bis(monoisopropylamino)fluorophosphine oxide	Pestox 15, Isopestox
Monuron	N-(4-chlorophenyl)-N'N'-dimethylurea	
phorate [b]	0,0-diethyl S-ethylthiomethyl phosphoro dithioate	Thimet [a]
Phosdrin [a]	dimethyl 2-,ethoxycarbonyl-1-methylvinyl phosphate	
Pyrolan[a]	1-phenyl-3-methyl-5-pyrazolyl dimethyl-carbamate	
schradan[a,b,]	bis(dimethylamino)phosphonous anhydride	
	octamethyl pyrophosphoramide	OMPA
Sevin	1-naphthyl N-methylcarbamate	7744
TEPP	tetraethyl pyrophosphate	
toxaphene	chlorinate camphene (67-69 % chlorine)	
Trithion[a]	0,0-diethyl S-p-chlorophenyl-thiomethyl phosphorodithioate	

CHAPTER 1

REVIEW OF LITERATURE

HISTORICAL INTRODUCTION :

The age old method of timber staining by implanting dyes into the growing tree through bore holes in the trunk, was based on the phenomena of systemic movement and distribution throughout the plant. This technique of colouring furniture wood led early entomologists to introduce compounds into the sap stream to render plant tissues toxic, or unpalatable to phytophagous insects.

In 1903 Demetiev observed that an introduction of barium chloride into the sap stream of apples gave control of woolly aphis. Potassium cyanide implantations were found to give good control of Icerya purchasi (Sandford 1914) Aphis rumicis on Vicia faba (Davidson 1924), and girdling and boring insects in elm and black locust trees. Moore and Ruggles (1915) reported failure in control of wood borers in oaks, following trunk implantations of potassium cyanide. Muller (1926) showed that pyridine implantations controlled woolly aphis on apple trees.

The introduction of toxins into the growing media was another avenue of application which was investigated as it was more convenient and less damaging to the plant. Muller (1926) showed pyridine and barium chloride implantations gave good control of aphids with no ill effects on the plant. Davidson and Henson (1929) revealed that pyridine when applied to the soil was absorbed and translocated in sufficient amounts by Vicia faba to be toxic to the aphids (Aphis rumicis). Hurd-Karrer and Poos (1936) showed that wheat grown in seleniferous soil was not attacked by aphids. Mason and Phillis (1937) and Neiswander and Morris (1940) claimed that selenium added to soils gave control of a number of insects on various plants. Fulton and Mason (1937) reported that rotenone when applied to bean leaves reduced the attack of Mexican bean beetle on subsequent growth. This report of systemic qualities of rotenone however has never been substantiated.

David and Gardiner (1951) maintained that, until 1947, no satisfactory systemic insecticide had been discovered and that potassium cyanide and selenium

compounds were the only compounds that had been seriously considered. Schrader and Kukenthal's work in Germany on evaluating the insecticidal activities of substances that were related to war gas (phosphorus fluorine compounds) showed that not only were many of them insecticidal but they exhibited excellent systemic action. Reports on Schrader and Kukenthal's work by Martin (1947) Martin and Shaw (1948) and Schrader (1948) initiated further research on the systemic properties of these chemicals. Reports by David and Kilby (1949), Ripper, Greenslade and Hartley (1950), David (1950b) and Hofferbert and Orth (1952), substantiated their findings.

Some of the more successful systemic compounds that were formulated by Schrader (1952 a & b) were schradan, dimefox, and Systox. McCombie and Saunders (1946) showed that sodium fluoroacetate was a very good mammalian poison. David (1950 a) evaluated sodium fluoroacetate in the hope that as it occurred naturally in the poisonous South African plant Dichapetalum cymosum it was possible that it might be tolerated internally by other plants. This was shown to be true and not only did the substance have good contact toxicity to Aphis fabae, but when it was applied to the roots and foliage of aphid infested beans it was shown to have a remarkable systemic effect. This substance is still recognised as a very efficient insecticide, but due to its high mammalian toxicity it has been discarded in most western countries, although it is still used in Japan.

DEFINITIONS :

With the sudden surge of systemic insecticides onto the market, uniformity in nomenclature and classification became necessary. Prior to 1952, teletoxic endotheropeutic, and systemic were some of the names in vogue to describe insecticides exhibiting this action. Ripper (1952) reports that by the Third International Congress of Crop Protection the name 'systemic' proposed by Martin (1947) was generally acepted. Bennett's definition of a systemic insecticide (Bennett 1949), 'a chemical substance which is absorbed and translocated to all parts of the plant rendering it insecticidal' was also accepted. Unterstenhofer's

modification of this definition includes the phenmena of translocation and storage for a limited time within the plant, has been generally accepted (Unterstenhofer 1950). The acceptance of a general definition was necessary as systemic action is not an 'all or none' phenomenon and most lipoid soluble organic insecticides can penetrate the root, leaf, and fruit. (Ripper 1957). Leaf penetration by non systemic insecticides was observed by Yoong (pers. Comm.1964).

Ripper (1952) proposed criteria for the classification of systemic based on the way they are broken down within the plant and the form they are ingested by the insect. These are :-

(a) Stable systemic insecticide compounds which are not broken down by the plant e.g. selenium and sodium fluoroacetate.

(b) Endolytic systemic insecticides in which the toxic compound is 98 % in its original form on ingestion by the insect, e.g. schradan, Hanane and Phosdrin. However, research by Casida, Chapman and Allen (1952) and Pietri-Tonelli and March (1954) have shown that schradan is only slightly converted to its active form in the plant and its selectively is due to enzymal activation within the insect. Ripper (1957) proposed a fourth group to suit this activation within the insect and named it zoometatoxic. This further subdivision seems to have met with limited acceptance by other workers in this field.

(c) Endometatoxic systemic insecticides are transformed partially or completely into other toxic substances. These metabolites are usually more toxic than the parent material. Systemics of this type are Systox, Thimet, and Disyston, (Fukuto, Metcalf, March and Maxon, 1955 ; Metcalf, Fukuto and March, 1957).

Further discussion on this important property of this group of systemics will be made under the section on breakdown.

ABSORPTION AND TRANSLOCATION :

The divergence of opinions on persistence and control recorded for specific insecticides both in the laboratory and the field may be explained by a quote from a review article on behaviour of systemics by Bennett (1957) : 'When using a systemic insecticide, the plant, instead of being a passive spray target, becomes an active participant in the subsequent processes, which governs the

efficiency of the application. The plant being a living system is in a continuous state of flux so that considerable variations occur. These variations exist not only between species but within species, at different times of the year, or at different times of the day, and also under varying environmental conditions of light, temperature, humidity, nutrition etc. It is probable that differences in some of these conditions are responsible for the variable results obtained with systemic insecticides reported in the literature." The efficiency of systemics is governed by three main processes, absorption, translocation and breakdown into non-toxic metabolites. Therefore any condition that influences any of these three processes influences the efficiency of the systemic insecticide.

Metcalf (1956) itemized the essential properties of systemics as :-

(a) Ability to penetrate through the roots, fruit, leaves or stems.

(b) Sufficient stability to allow the insecticide to exist as a toxin long enough to render the plant insecticidal.

(c) Sufficient water solubility to allow the insecticide to move with the transpiration stream. However this property in the light of more recent information could be amended to read - that sufficient water solubility is needed either by the parent material or its toxic metabolites to enable these toxins to move in the transpiration stream. (Metcalf et al. 1957)

Systemics seem to be confined to two main chemical groups of insecticides :

(a) Organo-phosphorus compounds, which comprise the majority of systemic insecticides.

(b) Carbamate or urethane group which is a relatively new group.

It would seem that the structure and resultant chemical properties of these two groups are better suited to systemic movement, compared with the chlorinated hydrocarbons, which have a very sparse representation in the list of insecticides, which exhibit true systemic action. Most of the organic insecticides can penetrate the roots, leaves and trunk, but the translocation of these substances appears to be largely a function of water solubility of the parent material or its toxic breakdown metabolites. DDT, dieldrin, aldrin, toxaphene and heptachlor are all very insoluble in water. Lindane on the other hand is also very insoluble, but when

applied to wheat seeds it was shown to have good systemic action. (Bradbury and Whittaker 1956).

Absorption.

There are several sites of application of systemic insecticides, e.g. foliar, trunk, seed and root. The advantages and characteristics of each determines which site should be used in relation to the problem to be tackled. Hurd-Karrer & Poos (1936) controlled Rhopalosiphum prunifoliae and red spider mites on wheat, when the soil was treated with selenium. Mason and Phillis (1937) substantiated these claims with cotton in the control of pink bollworm and cotton-stainer, while Neiswander and Morris (1940) working with roses and tomatoes found control of red spider mite was effected when sodium selenate was added to the soil or nutrient solution.

Schrader (1952b) showed that bis (2 fluoroethyl) acetal and bis (2 fluoroethoxy-ethyl) acetal when applied in a solution to the roots of grapes, controlled caterpillars, aphids, and Phylloxeria vitifoliae. Subsequent work by David and Gardiner (1953), Metcalf, March, Fukuto and Maxon (1954) and Bradbury and Whittaker (1956) have revealed that root absorption in comparison to foliar absorption was a much less selective avenue of absorption. The latter authors showed that BHC was systemic in wheat when applied to the seed but showed no systemic activity after foliar application. This was thought to be due to the difference in structure of the two organs concerned with absorption. Roots have a specialised structure for absorption while leaves possess a wax cuticle which acts as a barrier to penetration, of extraneous material.

Variations in the amount of insecticide taken up by the plant roots were shown to be due, in part, to the physical properties of the growing media to which applications were made, and the water solubility of the respective insecticides. Reynolds and Metcalf (1962) observed that plants treated with soluble insecticides applied to the soil were rendered toxic to aphids in two days, while those treated with phorate, an extremely water-insoluble insecticide, did not become toxic to

aphids until two weeks after the application. This delay was thought to be due to slow penetration of the soil resulting in delayed contact with plant roots. David (1951) showed that five to ten times as much schradan accumulated in the leaves of test plants grown in sand compared with test plants grown in soil. Tietz (1954) working with the thiono isomer of Systox concluded that the largest amount of insecticide was absorbed from a water medium, less from sand, and the least from soil. Davidson and Henson (1929) using pyridine to control Aphis fabae on Vicia faba showed that a higher concentration was required for control on soil compared with sand. David (1951) using 32P schradan reported that less was taken up by the plant from soil than sand as the insecticide was absorbed by the top layers of soil and could not reach the plant roots. Wallace (1951) showed that part of the reason for greater absorption from sand may be due to the fact that plants grown in sand have a more extensive root system. Organic matter content of the soil has been shown to be of great importance in retaining and binding insecticides within the soil. Edwards, Beck and Lichenstein (1957) reported that extracts from soils treated with aldrin and lindane, when subject to a fly bioassay, gave a good correlation between percentage killed and the organic matter content of the soils. This indicated the propensity of organic matter to bind soil applied insecticides.

Poor control of root maggot in rutabaga was thought to be correlated with high organic matter of the soil, (Forbes and King 1957). Casida, Chapman and Allen (1952) observed that more organic matter and finer particles of soil gave a reduced root absorption on schradan by peas, as indicated with a bioassay of third or fourth instar nymphs of Macrosiphium pisi.

Getzin and Chapman (1960) showed that the binding of insecticides by soil fractions was not only affected by composition of the soil, but also by the chemical structure of the insecticide itself. Schradan was found to be a reasonably efficient systemic when applied in sand, but was not efficient when applied to soil. Phosdrin gave 14 days protection to peas from the pea aphid when added to sand, but only two days when added to soils. This was thought

to be due to increase in hydrolysis, and volatilisation of the insecticide, when applied to soils. Phorate and demeton were shown to be less persistent in loam soils than in sand, and even less in soils in which there was a large organic matter content. This decrease in persistence was considered to be due to adsorption and binding of the insecticide by soil colloids. Phosdrin and schradan may also be rendered ineffective by this, but probably to a lesser extent, as the main avenue of loss with these insecticides seems to be in the vapour phase. Isolan, a carbamate, showed different trends to the organophosphorus insecticides in that it was absorbed in greater amounts by clay loams, than muck. The above authors thought that the high cation exchange capacity of the organic matter makes it a more effective binding agent for the organophosphorus insecticides compared with carbamates which do not contain a phosphorus atom in their molecule.

Bennett (1957) puts the variation of absorption of insecticides in sand, water cultures and various types of soil as being due to:-

1) Greater affinity of insecticides for adsorption and binding with soil colloids.

2) Varying contact of the root with the insecticide within the soil.

In view of other quoted work the second factor may be a result of the first, as well as a mechanical factor.

Selective absorption by the roots of some insecticides has been reported by various authors. David (1951 and 1952) showed Vicia faba roots selectively absorbed dimefox from solutions but from similar solutions schradan was rejected, showing that absorption of the two chemicals may differ. Tietz (1954) working with the thiono isomer of Systox and using the same test plant found that it was selectively absorbed by the roots on the first day and then uptake became constant, corresponding to the volume of water taken up by the plant. Bradbury and Whittaker (1956) found that C 14 labelled benzine hexachloride in solution, was selectively absorbed by wheat seedlings, and after 1-2 weeks 50-60 ppm were found in the seedlings from a solution with an initial concentration of 7-8 ppm. Casida et al. (1952) reported that with 32P schradan if the plant was deficient in phosphorus,

the uptake of schradan was enhanced, but conversely, if more phosphorus was added to the soil solution the amount of schradan taken up fell. There would appear to be a competitive effect for absorption, by plant roots.

Hacskaylo, Lindquist and Davich (1961) found no difference in the amount of dimethoate absorbed by root in phosphorus deficient solutions, although leaves contained less insecticides due to a decrease in respiration. Tietz (1954) recorded a rapid absorption of Systox by the root on the first day, gave an initial accumulation within the root, which was slowly disseminated to other parts of the plant. This phenomenon led Tietz (1954) to propose the ultra filter lipoid theory of absorption, whereby, due to the lipophilic nature of Systox it is absorbed by the lipoid components of the cell wall of the root hairs. As a result of David's (1951 and 1952) and Tietz's (1954) work Ripper (1957) suggested two processes may be involved in root absorption of insecticides. These are as follows:

1) Absorption may take place by the passive movement in the transpiration stream through the roots and into the shoots. The amount taken up by this system will be proportional to the uptake of water under constant conditions. This may be the mechanism for water soluble substances. Crafts and Yamaguchi (1960) and Crafts (1961) observed that most compounds that are water soluble are absorbed by the root and translocated.

2) An accumulation of insecticides within the root tissues to higher concentrations than the surrounding solution.

Crowdy, Grove, Hemming and Robinson (1956) investigating the movement of griseofulvin, an antibiotic within the plant, noted that two factors were involved in the absorption of this substance from the soil.

1) An active entry into the root which was inhibited by metabolic toxins.

2) A prolonged uptake linearly related to transpiration which has been shown to be a passive process as it is not affected by respiratory inhibitors, sodium azide and dinitrophenol.

This active process of uptake was observed by Tietz (1954) when excised roots of <u>Phaseolus vulgaris</u> were placed in solution of .01% of the thiono isomer of

Systox. Tietz (1954) observed that roots placed in this insecticide solution absorbed a much greater quantity of water than the control. This increased water absorption returned to normal in 8 to 15 hours. The reason for this was put down to one of two factors:-

a) An increase in respiration.

b) A direct effect on the permeability of the cell wall.

Crafts and Yamaguchi (1960) have shown herbicides accumulate in the roots of some plants very rapidly (dalapon and monuron) and the release of these substances into the xylem takes place at varying rates. Crafts (1961) thinks that uptake by the root and migration into the xylem is a passive process through the root hairs and cortical cells in the primary region of the root, behind the root tip, and by migration through the symplast (continuous interconnecting protaplasm) and into the stele and the apoplast (non living cell wall of the xylem tissue).

Translocation.

Conclusions of translocation and distribution of insecticides within the plant have been drawn largely from observations of biological effectiveness and failure.

It has generally been established that soil applied herbicides, fungicides and insecticides move in the transpiration stream in the xylem (Wedding and Metcalf, 1952; Bennett and Thomas, 1954; Tietz, 1954; Crowdy and Rudd-Jones, 1954; and Yamaguchi and Crafts 1958). Crafts (1961) in a review article concluded that there was little doubt that transport after soil application is in the xylem. Tietz (1954) showed that turbulent conditions increase the rate of uptake of 32P Systox thiono isomer from 80-90 centimeters per hour to 1.20 meters per hour. Bennett (1957) showed the rate of uptake of 32P dimefox in Salix viminalis was 11cm per hour, while Wedding and Metcalf (1952) using 32P schradan measured the rate of uptake in Phaseolus vulgaris at 17-58 cm. per hour. It has been observed that a restriction of transpiration prevented dimefox being given off from the leaf or stopped it reaching the leaf, (Bennett, 1957). Low temperatures

and high humidities reduced the rate of uptake of dimethoate over a period of 24 hours in comparison to plants at similar temperature and lower humidities. Similar trends were also shown at high temperatures. Parencia et al. (1957), Tietz (1954), Reynolds et al. (1957), utilizing different plants, demonstrated that the phytotoxic symptoms of burning occurred on the margins of the leaf where conducting tissues end in parenchyma tissue, giving a damming effect. Radio-autographs corroborate this peripheral accumulation of insecticide, (Metcalf and March, 1952: Metcalf et al. 1954 ; and Metcalf, Stafford, Fukuto and March 1957). Hacskaylo, Lindquist and Davich (1961), showed that the uptake of dimethoate was less, in a solution deficient in phosphorus compared with a normal solution. This was assumed to be due to a reduction in transpiration caused by a phosphorus deficiency. Tietz (1954) showed that ringing plants had no effect on the upward travel of insecticides from the ground. Lateral movement within the plant has been shown to be limited (Tietz 1954; Hanna Judenko and Heatherington 1955). Hanna et al. (1955) observed, unless trunk implantations or soil applications were applied evenly around the trunk of cocoa trees, then control of mealy bugs was only registered directly above the site of application.

The efficiency of systemic insecticides is governed to a large extent by distribution within the plant. Metcalf et al. (1952 and 1954) showed that distribution of dimefox thiono isomer, and schradan when topically applied to the based of lemon seedling stems were distributed throughout the plant. Reynolds et al. (1957) working with cotton, alfalfa, and sugar beet grown from insecticide treated seed, observed that the resulting seedlings contained a higher concentration of insecticide in the cotyledons, where it seemed to be stored and not redistributed. Cotton cotyledons contained 5 to 14 times as much insecticide as the rest of the plant.

Hacskaylo, Lindquist and Clark, (1961) and Hacskaylo, Lindquist and Davich (1961) showed that dimethoate and phorate accumulated in cotton leaves under enviromental conditions which favoured transpiration. Applications of these insecticides were made to the soil as granules.

One of the limiting factors in translocation is water solubility. Crafts (1961) states that most compounds that are water soluble are taken up by the plant root. Ferguson and Alexander (1953) showed an interesting gradation of systemic properties of dimetan, Pyrolan and Isolan. The first two are only slightly water soluble compared with Isolan, and do not accumulate in the upper parts of the plant. Isolan is a strong systemic, whether root absorbed or foliar absorbed. The difference is thought to be that the first two are not taken up fast enough by the root, due to their low solubility, to keep pace with guttation and breakdown. Metcalf, Fukuto and March (1957) observed that the oxidative metabolites of phorate and Disyston are more water soluble than the parent material, and it is thought that they are converted to this form in soil or root prior to translocation.

BREAKDOWN AND STORAGE WITHIN THE PLANT AND LOSSES FROM THE SOIL FOLLOWING SOIL APPLICATION OF INSECTICIDES.

The metabolism of insecticides within the plant can be divided into two classes:-

(a) Oxidative reactions by which the parent material and the metabolites from it, are usually activated to a more anti cholingenic substance.

(b) Hydrolytic reactions by which process the insecticide is converted into non-toxic metabolites.

Oxidation to a more powerful toxin takes place only with endometatoxic insecticides such as phorate, Metasystox, Systox and Disyston. Endolytic insecticides such as Phosdrin and dimefox are not oxidised to any great extent within the plant, although Metcalf et al. (1952) showed a small fraction of schradan was oxidised to a more powerful anti cholingenic compound. By definition endolytic insecticides are 98% present in their original form at the time of ingestion by phytophagous insects. Stable compounds, such as sodium fluoroacetate and selenium are not decomposed to any great extent in the plant. Kearns (1963) points out that phosphorothioates (Disyston and phorate) are not initially good in vitro

cholinesterase inhibitors, but are oxidised in the plant and insect to their corresponding sulphoxides, sulphones and phosphates which are toxic. Although these compounds are more anti-cholingenic they are unstable so are of little practical use in the field. Fukuto et al. (1955), Metcalf, Fukuto and March (1957) showed similar reaction paths within the plant for phorate, Systox and Disyston. The parent material or active metabolite after entering the plant may be oxidised or hydrolised. If the insecticide is oxidised it is converted to the corresponding sulphoxides and sulphones.

Oxidation of phorate and Disyston parent materials to the corresponding sulphoxides and sulphones results in a corresponding increase in anti-cholingenic properties, due to the fact that oxidation increases the polarity of the resultant metabolites. On the other hand with increased oxidation water solubility increases and translocation is more rapid. The presence of a P = O bond in which phosphorus is more electrophilic than in a P = S bond gives a more potent anti-cholingenic compound. The latter form is more unstable, and instability increases for both compounds with So and So_2 substitutions on the side chain. These oxidative changes may take place very rapidly. Metcalf, Fukuto and March (1957) showed that with Thimet and Disyston in cotton leaves the change may take place within two hours. Phorate and Disyston are converted by hydrolysis to non toxic metabolites. Mühlmann and Tietz (1956) suggested that the final product of hydrolysis in the plant is orthophosphoric acid which is resynthesised to phospholides, especially lecithin. Heath (1961) is of the opinion that Disyston and phorate follow a very similar process in degradation.

The speed of the oxidative and hydrolytic reactions are dependent on :-

(a) The type of plant. Heath, Lane and Llewellyn (1952b) working with schradan concluded that hydrolysis did not vary with crop species. However further work by Thomas and Bennett (1954) showed there was a marked difference in schradan decomposition, and beans broke the compound down more rapidly than Coleus. Metcalf, Reynolds, Winton and Fukuto (1959) showed that at 70°F. Disyston was hydrolytically decomposed 2-3 times as fast in

lemon as in cotton.

(b) Rates of oxidation are dependent on the range of environmental temperature, and Metcalf et al. (1959) working with Disyston claimed that an increase in temperature between 57°F.-100°F. increased the rates of oxidation in cotton. For every 10°F. rise in temperature, the oxidation rate increased 1.9 times.

(c) Season and stage of growth was shown to vary the rate of schradan decomposition. Heath et al. (1952a) observed that plant metabolism was more rapid in summer, in young quickly growing plants.

(d) Bennett and Thomas (1954) showed that a pretreatment of light increased the rate of decomposition of schradan which was maintained after the withdrawal of the light source. Darkness on the other hand, slowed the rate of decomposition.

(e) Some chemicals lend themselves to quicker breakdown than others, e.g. selenium compared with dimefox.

Other avenues of loss from the plant that are important are, guttation, leaching from the leaf and recretion from the roots back into the soil. Guttation was shown to increase with increased turbulence. Artificial rain increased losses from the leaf due to a leaching effect, (Tietz, 1954). Volatilisation from the plant of Phosdrin, dimefox and schradan was also shown to be an important avenue of loss from plant, (Bennett et al. 1954 and Arthur and Casida 1958).

The equilibrium between uptake from the soil and hydrolytic decomposition within the plant determines the level of toxins within the plant. With soil applied insecticides the above avenues of loss and factors affecting loss, are not as important as with foliar applied insecticides. Persistence of soil applied insecticide relies upon uptake from the reservoir of insecticides within the soil or on the seed coat. Bardiner (1964) showed that when wheat plants which had arisen from seeds treated with insecticide, had the seed casing detached from the seedling and were re-sown in non-treated soil, they quickly lost their toxicity.

After uptake of insecticides by the roots, insecticides accumulate in the peripherial margins of the leaf, (Tietz, 1954; Metcalf et al., 1952; Metcalf

et al., 1954; Metcalf, Fukuto, March and Stafford, 1956).

Factors that effect the persistence of insecticides in the soil are:-

(a) Volatilisation. This is a very important avenue of loss but depends on the vapour pressure of the chemical. Getzin and Chapman (1960) showed that Phosdrin is lost from the soil largely by this method. Harris and Lichtenstein (1961) also observed loss of phorate from the soil in the vapour phase. These authors found that soil texture, soil structure, soil temperature, soil moisture level and turbulent conditions above the soil effected vapour losses of aldrin from the soil.

(b) Chemical hydrolysis and metabolism by soil microflora. Heath (1961) reported that dimefox is degraded to its ionic product by natural soils. The rates of decomposition were found to be equal to the hydrolysis rates of soil calculated from their PH values. Mounter, Baxter and Chanutin (1955) named nineteen micro organisms capable of metabolising di isopropyl dialkyl fluorophosphate to liberate the fluoride ion. Large differences in activity were observed with various micro organisms. This reaction was found to be potentiated or inhibited by Mn++ Ca++ or Mg++, depending on the organism. Wackers (1955) showed a green alga was able to store Systox internally. Ahmed and Casida (1958) showed that phorate is hydrolysed by two bacteria Pseudomonos fluorescens and Thiobacillus thiooxidans, the latter being the more efficient converter. The sulphide analogues were more prone to hydrolysis than the corresponding sulphones and sulphoxides. These bacteria abound in soils. T. thiooxidans is more active in soils treated with sulphur containing fertilizers. P. fluorescens has an important role in the decomposition of lignin (Waksman, 1932). Tarulapsis and Chlorella species showed the ability to oxidise various organophosphates.

(c) Leaching. The extent of leaching has been observed to be very small. The binding of insecticide to the soil particles seems to be more important in depleting insecticide deposits. Hacskaylo, Lindquist and Clark (1961) observed that 1 to 7 weeks after planting, 70% of the applied phorate was in the top 1.5 inches of soil. Reynolds (1957) reported that poor control under irrigation was probably a result of leaching. Zaki and Reynolds (1961) found

that phorate, Systox and dimethoate formulated on attaclay leached less than the same chemical on vermiculite. The relative rates of leaching of the insecticides were as follows: dimethoate > Systox > phorate, and the relative rate of leaching in various soils were in the following order: Loamy fine soil > fine sandy soil > silty loam > clay soil.

(d) Adsorption and binding of insecticide by the soil depends on the cation exchange and the organic matter of that particular soil, (Casida et al. 1952; Edwards et al. 1957 and Getzin et al. 1960).

DISCUSSION ON THE PROPERTIES OF SYSTEMIC INSECTICIDES.

Advantages of systemic insecticides.

Systemics have many advantages when compared with contact insecticides. Their most important feature is their ability to kill concealed feeders due to their penetrating powers. Hanna et al. (1955) showed that mealy bugs on cocoa trees, protected from contact insecticides by carbon tents, which were constructed by ants (Crematogaster species), were killed by root or trunk application of dimefox. Brown (1960) recorded success in control of hessian fly maggots with phorate granules. Ripper (1957) reports that systemic insecticides gave some control of Phylloxeria vitifoliae. Hirchmann (1953) has shown that foliar nematodes have been controlled with systemic insecticides. Schread (1959) recorded good control of leaf miners with soil applied phorate.

Systemics have the ability to protect young foliage which has not appeared at the time of application. This ability is of course tied up with translocation and persistence which is dependent on the site of application and type of insecticide used. Phosdrin and dimefox are non-persistent systemics when applied to the foliage and are very quickly lost by various reactions (Ripper 1957). Hanna et al. (1955) reported that dimefox applied to cocoa trees as a trunk implant gave up to six months protection from mealy bugs. Another advantage of systemic insecticides is that they have four sites of application which give different persistence and varying degrees of specificity, hence the site may be chosen in relation to the action desired.

Advantages and disadvantages of various sites and methods of application of systemic insecticides.

(1) Distribution patterns of the insecticide within the plant may vary with application sites. Tietz (1954) showed that there was very little movement of insecticide within the leaf, and only when application was made to the basal part of the leaf was movement observed. The direction of this movement was to the apical part of the leaf. Translocation within the plant after foliar application was very poor, and only a very small fraction of the insecticide was translocated at all. Tietz (1954), Bennett and Thomas (1954), David (1952) and Metcalf et al. (1952) showed that with 32P Systox or schradan movement upwards after foliar application was greater than the downward movement. Thomas, Bennett and Lloyd-Jones (1955) observed that translocation of 32P Systox from the leaf in comparison with that from the root was slower and very much reduced. Tietz (1954) stressed the importance of obtaining as near complete coverage as possible with foliar systemic sprays in order to render all the plant toxic. Translocation following seed treatment does not seem to differ appreciably from that found after root application (Mitchell, Smale and Metcalf, 1960). David and Gardiner (1955) showed that broad bean plants originating from seed soaked in Systox thiono isomer possessed high concentrations of insecticide in stems and seed. Reynolds and Fukuto, Metcalf and March, (1957) observed that alfalfa, sugar beet and cotton originating from insecticide treated seed held high concentrations in the cotelydons where it was stored and not re-distributed throughout the plant. This concentration and storage were also evident with soil drench and granular applications. Reynolds et al. (1957) and Bardiner (1964) concluded from tests on the entry of insecticide into the plant following seed coating, that the compounds appear to enter largely through the roots. Trunk or stem applied insecticides follow similar paths to root applied, (Hanna et al. 1955; Metcalf et al. 1954; and Metcalf, Stafford, Fukuto and March, 1957).

(2) Ground and trunk implantations, stem applications and seed treatments are

much more persistent than foliar applications. With foliar application waste and losses may be very high due to the volatility of the chemical, the temperature, and the nature of the plant surface, (Tietz 1954, and Bennett and Thomas 1954). Heath, Lane and Llewellyn (1952) have recorded 50% loss of schradan from brussel sprouts after foliar application. Tietz (1954) observed that varietal difference in retention of the insecticide on the plant following foliar application, was quite large and that more was retained on the hairy surface of primula in comparison with the smooth surface of cyclamen. Tietz (1954) and Bennett and Thomas (1954) showed that more of the insecticide was retained if treatment was made to the under surface of the leaf, and also that young leaves on most plants absorbed more as they have a more permeable cuticle. With soil applied or trunk implanted insecticides the site of application provides an area where a store may be held from which the plant may draw. With foliar applied insecticides the amount that the leaf can hold is very small. Depending on the size, age and variety, leaves vary widely as efficient spray targets. With foliar spraying, the site of application is open to turbulence and rain before entry into the plant is made. Wind and temperature affect volatilisation losses, and rain affects leaching from the leaf. Light, temperature and humidity affect the amount of insecticide absorbed after foliar application. (Tietz, 1954; Heath et al. 1952, and Bennett and Thomas 1954). Jeppson, Jesser and Complin (1952) showed that with lemons, 1.5 ozs Systox per tree applied to the trunk gave at least six months' control of citrus mite, through the spring and summer months. The maximum duration of control that spray applications gave under the same conditions was very much reduced, although up to six months' protection was recorded in seasons of low temperatures which retarded volatilisation and the lowered intensity of the sun decreased photo-chemical deterioration. Cook, Walker and Featherston (1963) and Pond (1963) observed that phorate and Disyston granules treated to the soil gave two to three months' protection from aphid infestation on potatoes. Long periods of persistence following

soil or seed application of insecticide have been recorded with a wide variety of plants by Reynolds et al. (1957), Parencia, Davis and Cowan (1957), Bishop and Burkhardt (1959), Bardiner (1960), Zaki et al. (1961) and Allen, Askew and Schreiber (1961).

(3) Work by David and Gardiner (1953), Tietz (1954) and Bradbury and Whittaker (1956) showed that foliar absorption of exogenous materials is more selective than absorption through the root. Tietz (1954) observed that penetration of the leaf cuticle was retarded if compounds were polar in structure.

(4) Systemic insecticides have been shown in general to be more specific than contact insecticides. This specifity or selectivity may be due to two factors:

(a) Physiological - this type of selectivity is caused by the insects' metabolic or nervous systems being relatively unaffected by this particular insecticide in comparison to those insects which are killed by the insecticide.

(b) Ecological selectivity is caused by the insecticide being positioned in such a place so that it comes only in contact with a certain group of insects. Because systemics are translocated most of these types of insecticides applied to roots and stems have this property of ecological selectivity.

Schradan does not kill coccinellids, syrphid larvae or most other predators either by contact or by feeding on poisoned aphids. (Ripper 1951 and 1956). Isolan also has a selective physiological action and is ineffective against red spider mites and beneficial insects, such as Anthocoridae and Chrysopidae species (Spindler 1955). Most materials such as dimefox, mipafox, Systox, phorate and Metasystox are efficient contact insecticides and kill most parasites and predators and may also kill larvae of Syraphidae and Coccinelidae feeding on poisoned aphid (Ahmed 1955, David et al. 1951 and Zattler 1951). Phorate and Metasystox applied as soil drenches, as coatings to the seeds, trunk implantations and granules to the soil gave ecologically selective control. (Reynolds, Anderson and Swift 1953, and Metcalf 1956). Selective control of insects was reported of trunk applications of insecticides made to citrus,

cocoa and coffee trees, (Bond, Hanna et al. 1955 and Jepson et al. 1952).

(5) Seed coating with insecticide has been shown to severely reduce sowing rates, due to the increase in size of each seed, (Lange 1959 and Reynolds 1958). Lange (1959) is of the opinion that where a severe reduction of sowing rate is caused by treatment of the seed then, in most cases, it is better to dispense with seed treatment and sow at heavier rates. By sowing at higher rates allowances are made for losses sustained from insect attack. A severe reduction in sowing rate may be further aggravated by an inhibition of germination caused by seed treatment, (Hacskaylo and Ranney 1961).

(6) In 1957 Broadbent, Burt and Nix estimated that the losses from machinery damage, through continual spraying, in some seasons just balanced returns from spraying. Pond (1963) showed that one application of phorate sown with seed was as economic as one spray application but was three times as persistent in control of potato aphids. This was also observed by Broadbent, Burt and Heathcote (1964).

Soil applied insecticides reduce time, expense and soil compaction compared with the several spray applications to give the same protection, (Pond, 1963). Machinery costs are higher for spraying but lower for granular application as granules can be sown through the planter.

However to offset these advantages is the fact that if seed or soil treatment has been carried out at planting and no insect attack appears in the district then money has been wasted.

(7) With soil applied systemics it is possible to protect emerging plants at a time when it is impossible to spray due to lack of foliage. This point is of vital importance in virus control, particularly in regard to potato leaf roll virus, (Broadbent, Heathcote and Burt, 1960, and Pond, 1963). Newly emerging seedlings are very susceptible to damage from insect and virus attack.

(8) With spraying the atomisation of poisonous substances presents a human hazard. Drift from spraying constitutes a further problem, (Barnes 1953, 1957).

P A R T I

THE PERSISTENCE

OF

PHORATE AND ISOLAN GRANULES

APPLIED TO SOIL

FOR

APHID CONTROL IN CEREALS

CHAPTER 2

REVIEW OF LITERATURE AND METHODS

INTRODUCTION

Experiments in Part I were set out for the express purpose of obtaining information on the persistence and efficiency of granular, systemic soil applied insecticides for the control of Rhopalosiphum padi (L.) on barley, wheat and oats. Some factors that may effect efficiency and persistence of phorate and Isolan were also investigated. From these glasshouse trials it was hoped to obtain some idea of the persistence and efficiency that could be expected from these two granular insecticides in the field.

REVIEW OF LITERATURE

Soil applied systemic aphicide granules depend for their efficiency and persistance on good soil stability, strong systemic action and slow release from the carrier. This release from the granule must occur in sufficient quantity to render the whole plant toxic to insects. Spring wheat crops in the North Island of New Zealand treated with 1,2, and 3 lbs active ingredients (a.i.)/acre of Disyston granules applied to the seed furrow with 2 cwt of superphosphate/acre gave poor control of cereal aphid. These negative results were considered to be due to the insufficient soil moisture which is essential for the release of insecticide from the granule, (Anon 1964). It has been shown that low water soluble granulated insecticides such as phorate and Disyston may give delayed or erratic control. Reynolds and Metcalf (1962) observed that phorate accumulated very slowly within the cabbage plant when granules were applied near the roots. Fourteen days elapsed after application before the plant became toxic to the cabbage aphid. In comparison some of the more water soluble insecticides that were treated in this manner rendered the plant toxic to cabbage aphid inside two days. This was thought to be due to faster diffusion from the granules into the root zone area and rapid uptake by the roots of the plant. Brown (1960); Burt, Broadbent and Heathcote (1960) and Doucette (1961) also observed that the water insoluble insecticide

phorate, when applied as side applications resulted in poor insect control. In comparison, efficient and persistent control was gained with similar treatment rates made to the seed furrow, (Brown, 1960; and Doucette, 1961). Burt et al. (1960) using phorate, Rogor, and phorate mixed with fertilizer, recorded persistent and efficient control of aphids on potatoes with Rogor and phorate mixed with fertilizer, but phorate applied on its own did not exhibit efficient or persistent control. It was thought that the low water solubility of phorate prevented the insecticide diffusing through the soil, which results in the localisation of the insecticide so that with root growth the insecticide becomes lost to the absorptive root zone area. When applied with fertilizer the insecticide covered an area beyond which the roots were unlikely to penetrate. Rogor on the other hand is 2.5% soluble in water and thus has the ability to diffuse through the soil into the root zone area.

Granular properties have a profound effect on the speed with which insecticides are released from the granulated base. Most of the investigations on the release of insecticides from various bases have been carried out in conjunction with mosquito larvae control in water. However insecticide release from granules placed in the soil follows the same principle of dissolution or displacement in/by water. Temperature, type of insecticide, solvent used for impregnation of granules, and the concentration of insecticide on the granular base all have a marked influence on the release rate of insecticides. The rate of release from the granule is for most bases inversely proportional to size.

Celite, vermiculite, attapulgite and montmorillonite granules are some of the more common materials used as insecticide bases which give a rapid release of parathion when placed in water. Calcium carbonate (calcite), Ental and Pyrax granules seem to release parathion more slowly. It is probable that granules which disintegrate in water give a faster release while non-disintegrating materials give a slower release of insecticides. Most clay type granules have been observed to disintegrate in water. Physical characteristics of granule carriers such as formation of colloidal suspensions, binding and absorptive forces of particles

acting on a specific toxicant, may greatly influence the release of toxicants.

Celite, attapulgite, vermiculite and montmorillanite have a high absorptive capacity (greater than 30%) while calcium carbonate, Ental and Frianite have a low moderate absorptive capacity (less than 20%). With the former group, insecticide is taken into the material like a sponge while with calcite the material is absorbed to the outside (Mulla and Axelrod, 1962).

Zaki and Reynolds (1961) reported that the properties of the granular base on which the insecticide is formulated is important in the resulting persistence of control. These authors observed that vermiculite granules released toxicants more rapidly than insecticide based on attaclay and gave less persistent control.

Reynolds and Metcalf (1962) are of the opinion that water soluble soil applied insecticides are not as persistent as water insoluble insecticides.

As has already been outlined in the general review of literature the number of factors that contribute to, and influence the persistence of soil applied insecticides is wide and varied. However soil type, soil moisture, soil temperature, the volatility of the insecticide and the chemical hydrolysis and microbial breakdown in the soil play an important role in determining the persistence of insecticide within the soil. As there are so many variables that determine the persistence, little information is derived from quoting other authors' work, as this period of protection given by soil applied systemic granules may vary with similar plants and dosages when subjected to varying conditions.

In general it is maintained by the manufacturers that the persistence of phorate's aphicidal properties on attapulgite granules when applied at rates of 1-2 lbs. a.i./acre in the seed furrow, ranges from 3-6 weeks, (Hall, pers. com. 1964; and Schroeder, pers.com. 1964). This period of protection from these rates of phorate is amply substantiated in the literature. Reynolds, Fukuto, Metcalf and March (1957) observed that 1 lb. a.i./acre of Thimet gave control of aphids on alfalfa for 4-6 weeks after sowing with seed. Bacon (1960) reported that phorate at 2 lbs. a.i./acre gave control of aphids on potatoes for 80-85 days after planting when applied in the soil with the seed tubers. Andres, Reynolds and Fukuto (1959)

obtained two months' control of cabbage aphid with phorate applications of 1 lb a.i./acre applied to cabbages and cauliflowers in the seed furrow. Phorate applied at rates of between 1.5-4 lbs a.i./acre beneath the seed tubers protected potatoes from aphids for three months. These observations were carried out over a number of years. Hacskaylo, Lindquist and Clark (1961) gained 9 weeks control of the cotton aphid on cotton, with rates of 1.7 lbs a.i. of Thimet/acre. De Pew (1961) however, was only able to secure two weeks control of the spotted alfalfa aphid with 1 lb a.i./acre applied to the seed furrow with alfalfa seed.

The most important factor that effects the persistence of soil applied granular systemic insecticides irrespective of environmental conditions, is the rate of application. Hopkins, Fye and Walker, (1959) observed that high rates of Thimet had a longer residual life than lower rates. 30 lbs a.i. of Thimet/acre gave more persistent control over a period of time than applications of 10 and 20 lbs a.i. of Thimet/acre. Savage and Harrison (1962) reported that 4 lbs a.i. of phorate/acre gave more persistent control than a 1 lb application rate, in controlling Myzus persicae in tobacco. 8 lbs a.i. of phorate/ acre was reported to give a longer period of protection to Easter lilies than 1,2, and 4 lbs a.i. application rates, (Doucette 1962).

The persistence of control resulting from Isolan granular applications is poorly documented and in fact no reference to this property in Isolan has been located.

METHODS

The general method used for determining the efficiency and persistence of phorate and Isolan was basically one of treating the soil in which the plant was growing, and exposing aphids to treated plants over a seven day period. The granular based insecticides used in all the following experiments were phorate and Isolan. Phorate was formulated as a 10 % granule on an attapulgite base with a mesh size of 18-36 B.S.S., while Isolan was formulated as a 5 % granule on a calcite base of 18-56 B.S.S. mesh size.

R. padi (L.) was chosen as the test organism for evaluating the persistence and efficiency of phorate and Isolan as soil applied systemic.

insecticides for aphid control. The choice of this species of aphid was made due to its economic importance as the vector of Barley Yellow Dwarf Virus in New Zealand and the fact that its graminaceous host plants are grown rapidly under glasshouse conditions.

Culturing Test Insects.

The form of aphid which was to be used for this bioassay created a problem. Obviously the apterous adults which are most plentiful on the cereal crops in the autumn and spring, were the ones in practice that control methods would be directed at. However, they are very hard to rear under high temperatures in the glasshouse. Growth cabinet observations showed that aphids when subjected to temperature within the range 15°C ± 1.6 were near optimum reproductive potential for the production of nymphs which develop into the apterous forms. When the aphid cultures were subjected to temperatures of over approximately 22°C. the production of apterae was very small and alate was very high.

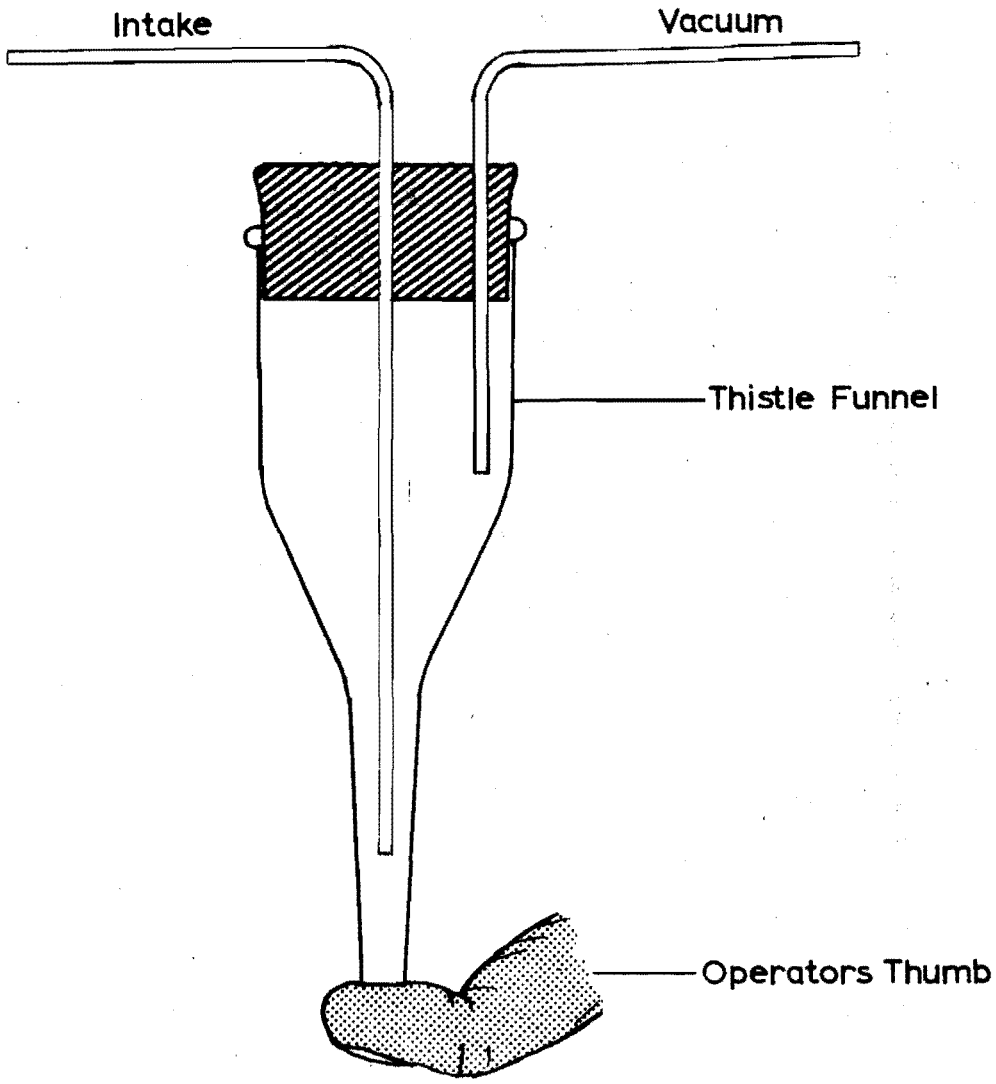
Aphids were cultured upon wheat, barley, oats and ryecorn. These cereals were sown in a 3-1 mixture of sand and field soil, in six-inch clay pots. When inoculating the plant it was imperative to have the plant at the right size, and not to inoculate with too many aphids, otherwise the plants collapsed. Under glasshouse conditions at 13°C-18°C inoculation of the plants was made two weeks after emergence. Plants were harvested for aphids at 6-8 weeks after inoculation, depending upon the size and progress of the colony. It was observed that the best results were obtained when the aphid colony was left undisturbed on the plant for the longest possible time.

Plant health was noted to be very important for good and rapid build-up of apterae. As mildew (Erisiphum graminus) is a problem of wheat and barley grown under glass, host plants for aphid cultures were alternated to avoid its build-up. Alternative cereals used which were not attacked by this variety of mildew were ryecorn and oats. Hilgendorf 61 strain of wheat was also used to alleviate this mildew problem as it was supposedly resistant. However under glasshouse conditions this resistance broke down. A factor of equal importance for mildew control was to avoid infesting test plants when inoculating with aphids as severe attacks of mildew

FIGURE 1

Modified aspirator used for handling aphids

MODIFIED ASPIRATOR FOR PLACING APHIDS IN CAGES



terminated part of Experiment 1. To avoid a re-occurrence of this, all aphids that were used to infest wheat or barley test plants were taken from oats or ryecorn culture plants. Ryecorn was better from the point of view of mildew suppression, whereas oat culture plants seemed to succumb to a variety of mildew.

Various cages were used to contain aphids upon culture plants. The first designed were cylinders, six inches in diameter, twelve inches high, enclosed at one end with gauze and possessing five, two-inch diameter holes evenly spaced around the walls. These were initially made from cellulose acetate. Unfortunately this substance proved to be toxic, not only to the plant which yellowed off at the point of contact, but also to aphids which did not build up in numbers on plants, covered with these cages. Perspex was used to replace this material and cylinders made from this to the above specifications gave none of these side effects. Trouble did however develop which eventually led to their replacement. This was humidity, which built up within these cages due to evaporation from the soil and plant transpiration. This high build-up of humidity caused the formation of water droplets on the walls of the cylinders in which some of the aphids drowned. Another facet of this high humidity was the rapid build-up of mildew which was favoured by this type of micro-climate. Nylon gauze hoods suspended from the roof of the glasshouse, which pulled down over the trays of inoculated culture plants contained the aphids without the accompanied humidity build-up. Cages were found necessary not only to contain aphids but to protect cultures against parasites and predators. Micromus tasmaniae Wlk., an aphid predator and Aphidius rapae Curt., an aphid parasite, gave trouble in cultures which were left uncovered.

Handling of Test Insects.

When test aphids were needed, plants on which the cultures were breeding were cut at soil level and shaken onto a plastic board which had been given a vigorous rub. This rub induced a static charge which made it very difficult for the aphid to move from the board.

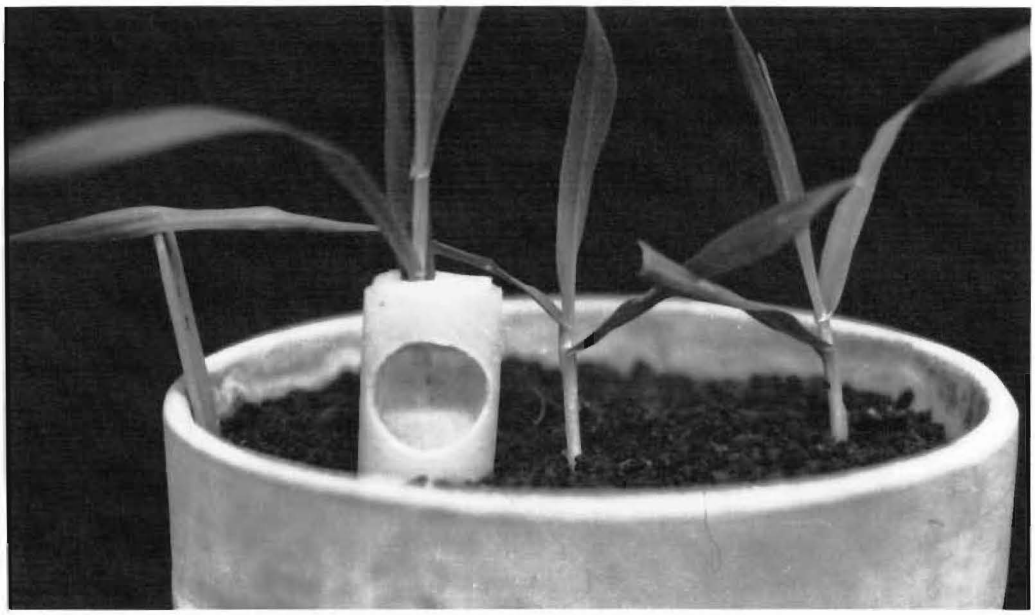
This method of removal from plants does not injure the mouth parts of R. padi (L.)

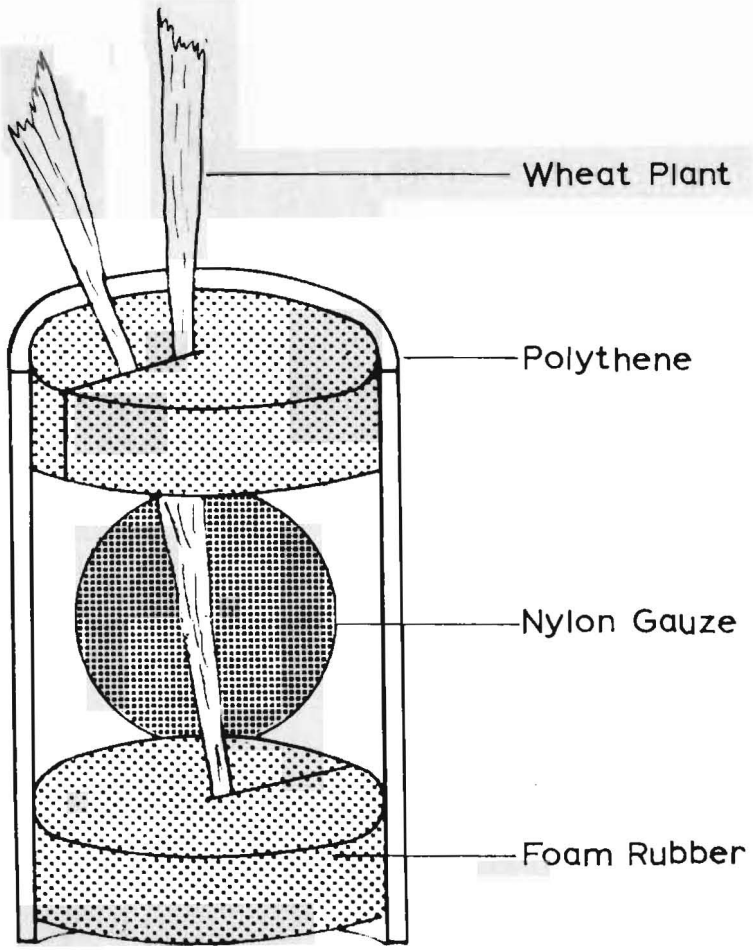
PLATE 1

Aphid cage used for caging aphids on treated plants

FIGURE 2

Construction of the aphid cage shown in Plate 1.





CONSTRUCTION OF APHID CAGE

as it does with other species of aphids. This was shown by taking survival rates of aphids which were caged on plants for three days after subjecting them to this treatment

From the board, the aphids were sorted for adult apterae. These were sucked up by means of an aspirator, made from a large thistle funnel in which the intake pipe terminated in the thin outlet tube of the funnel, as shown in Figure 1. When a vacuum was applied to the suction pipe the aphids was swept through the intake pipe and dropped into the narrow outlet tube of the funnel. The vacuum was created in the funnel by sealing the outlet tube with the operator's thumb. By removing the thumb and gently tapping the funnel the aphids dropped out into the cage which was to house them on the test plants.

By means of this simple piece of apparatus, over 1,000 aphids could be caged on plants in two hours, which was much faster than by using a camel hair brush.

The cages (see Plate 1) that were designed to hold aphids on plants, were made from white $\frac{3}{4}$" polythene tubing which was one of the few materials which was available and not toxic to aphids. $1\frac{3}{4}$" lengths were cut from the tubing and a $\frac{3}{4}$" cork-borer was used to bore a hole in each side of the wall. Nylon gauze was stuck over the inside wall of the hole with a waterproof glue. With a $\frac{3}{4}$" cork-borer, discs were cut from $\frac{1}{2}$" foam rubber. A radial slit was cut in each disc, (see Figure 2). The test plant foliage was led through this trap and plugged at the bottom with a disc. Foliage was fitted into the slit cut into the foam rubber. Aphids were dropped in through the top and this was also closed by another disc. This left a $\frac{3}{4}$" cavity in which the aphids were housed.

The advantage of this type of trap was that injury to the plant was negligible, providing that the foliage was not jammed against the walls of the container when plugged with sponge rubber. Traps could be placed anywhere on the plant. If aphids were trapped on the leaves, the trap was fixed by a drawing pin to a wooden spatula which was implanted in the soil. Plants could be spray watered without causing injury to the trap or the enclosed aphids. Light penetration into the trap was not hindered to any great extent by virtue of the large aeration holes which enabled diffused light to penetrate the container. The only part of the plant which was without light was

that part enveloped by foam rubber. Chlorosis of the area was avoided by moving the trap slightly up the stem during the following incubation period.

Assessment of Bioassay Results.

After the plants had emerged and were at a well developed first true leaf stage, they were infested with five adult apterous aphids. These were maintained on the plant for seven days, and the establishment of nymphs was recorded as nil or positive.

To gain information from these experiments, an assumption was made. This was, that if one replicate out of five within a treatment recorded a positive result for aphid establishment, then that treatment was designed as inefficient. This assumption was made for two reasons. Firstly, that for Barley Yellow Dwarf Virus control a 100% control of aphids is needed (Smith, pers.com. 1963), and secondly, it was thought that the application to seed furrow and soil was more accurate than could be hoped for in the field.

For plants resulting from seed sown with phorate granules within the furrow it was shown that plants may give positive results for one incubation period but negative results in the subsequent test. This was thought to be due to the movement of insecticide through the soil into the absorptive root zone area, or conversely, the movement of the root into contact with a small deposit of insecticide. To counter this, plants were tested for another week after they were deemed non-toxic. If a positive result was substantiated, plants were recorded as positive on the date that this was first observed. This recording was used for all persistence trials except for side and surface placement of insecticide where all results were taken, to observe if and when the plant absorbed the insecticide.

All glasshouse experiments were laid out in randomised blocks. The size of these blocks was dependent on the number of treatments, but the smallest possible block size was used in all experiments. Trials carried out in growth cabinets were set out at random and as in glasshouse trials were shifted every two days.

In this thesis experiments were carried out in soil held at approximately 60%

field capacity in order to eliminate one of the more important variables effecting efficiency and phytotoxicity of soil applied insecticides. This was achieved by estimating the water holding capacity of the potted mixture by Coutts' modified Keen-Rackowski box technique, (Coutts 1930). A calculation of pot plus soil weight at 60% field capacity was worked out making allowance for pot weight. Unless otherwise stated watering was carried out every day or second day depending on glasshouse conditions. This held the soil within the 55 to 65% field capacity range.

C H A P T E R 3

EXPERIMENTAL DESIGN, RESULTS AND DISCUSSION

EXPERIMENT 1. PERSISTENCE OF INSECTICIDES.

DESIGN.

This experiment was laid down with the purpose of determining the persistence of insecticides at various concentrations and applied to various species of cereals. It was also used to give a preliminary survey of the phytotoxic effects that phorate and Isolan might have on the cereal species, (see Part III, Experiment 1). Hilgendorf wheat variety was used in all experiments in an effort to curb mildew build-up. The varieties of oats and barley that were used were Garton Onwards and Kenya. These species were sown at 1½ bushels/acre, an approximation was three seeds per pot.

One hundred and sixty-eight 6" clay pots were filled with field soil which had been sieved through a ¼" wire mesh screen. This procedure was standard practice in preparing soil for potting. Soil was mixed in a 5-1 ratio with sand, to give better drainage and prevent consolidation resulting from surface watering. Phorate and Isolan were sown within the seed furrow at 1, 2 and 3 lbs. These rates were based on pound of active ingredient/acre. Each treatment rate had eight replicates. A control also possessing eight replicates was added as criteria for aphid establishment.

Two weeks after sowing, one plant was chosen at random within each pot, and infested with five apterous adult aphids. Only five replicates out of the eight which were initially set out for each rate of insecticide were used. After germination three of the pots from each treatment were withdrawn from this experiment. Aphids for each test were incubated on the plant for 7 days at which time establishment of nymphs was noted. This procedure was carried out in all experiments in which this type of bioassay was used.

RESULTS.

The tests on barley plants within this experiment were disbanded after six

weeks due to a severe infestation of mildew. A similar infestation on wheat plants after eight weeks necessitated the termination of the whole experiment. The results of the bioassay carried out in this experiment are given in Table I. All rates or applications are given in lbs. of a.i./acre.

Table I. The number of replicates of 3 plant species supporting aphid colonies at intervals after infurrow treatment with insecticide.

INSECTICIDE		Isolan					Phorate				
No.Weeks after Treatment		4	5	6	7	8	4	5	6	7	8
Cereal	Rate/acre										
Barley	1 lb. a.i.	1	2	4	-	-	0	2	3	-	-
	2 lb. a.i.	0	0	2	-	-	0	1	2	-	-
	3 lb. a.i.	0	0	0	-	-	0	0	0	-	-
Wheat	1 lb. a.i.	0	1	2	3	5	0	0	2	3	4
	2 lb. a.i.	0	0	1	1	3	0	0	0	2	3
	3 lb. a.i.	0	0	0	1	2	0	0	0	0	0
Oats	1 lb. a.i.	0	2	4	4	4	0	0	1	2	3
	2 lb. a.i.	0	0	1	3	3	0	0	0	0	2
	3 lb. a.i.	0	0	0	1	2	0	0	0	0	0

N.B. 0 = nil
- = not done

It would appear from Table I that the difference of persistence of Isolan under different species of cereals is negligible. With phorate under barley it was slightly less persistent than under the other two cereals. However in an experiment of this type which possesses so many variables, this fact would require substantiating with further trials before it could be taken as valid. Two things that did stand out from the above table are that phorate was consistently more persistent than Isolan under these conditions and that persistence increases with increased rates of application.

Perhaps the most important fact exhibited by this table was that these insecticides applied in this manner under these conditions gave at least one

month's protection from aphids after sowing. The exception in Table I was Isolan treated soil at 1 lb. a.i./acre under barley.

EXPERIMENT 2. EFFECT OF TEMPERATURE ON PERSISTENCE.

DESIGN.

The purpose of this experiment was to see if any difference in persistence developed when wheat growing in treated soil was subjected to two extremes of temperature.

The temperatures chosen for the growth cabinets in which the experiment was run were 24°C and 13°C. Eighteen pots were subjected to each temperature. Three treatments were laid down within these pots each having six replicates. Phorate and Isolan were applied at one rate of 2 lbs. a.i./acre and a control was incorporated. Five inch square plastic pots were used in this and all subsequent experiments. Sieved soil was mixed with perlite at a ratio of 6:1. Perlite was used in preference to sand in all following experiments in this ratio with soil as it gave better drainage and was found to be better in preventing consolidation. Watering was carried out every day for plants at 24°C and every third day for the 13°C cabinet. Plants under 24°C were tested 2 weeks after sowing and those under 13°C 3 weeks after sowing. Intervals of testing are similar to those described in Experiment 1.

RESULTS.

Table II. The number of replicates recorded at weekly intervals showing aphid establishment.

Temperature	13°C					24°C					
No. of weeks after treatment	3	4	5	6	7	2	3	4	5	6	7
Insecticide Phorate 2 lbs. a.i./acre	0	0	0	0	0	0	0	0	1	3	6
Insecticide Isolan 2 lbs. a.i./acre	0	0	0	0	2	0	0	0	3	4	5
Control	6	6	6	6	6	6	6	6	6	6	6

After five weeks at 24°C phorate and Isolan treatments gave inefficient control. In comparison similar treatments under 13°C gave effective control until 7 weeks at which time the experiment was stopped. No difference was noted between phorate and Isolan at both temperatures. This short persistent life at 24°C would suggest that high temperatures enhanced breakdown within soil or increased the volatilization of the insecticide from the soil.

EXPERIMENT 3 PERSISTENCE AND EFFICIENCY IN RELATION TO PLACEMENT OF INSECTICIDE AND SEEDING RATES.

DESIGN

Two separate trials with wheat are described under this heading, as both were carried out simultaneously. Trial (a) was laid down in order to compare the persistence and efficiency of side and infurrow applications of phorate and Isolan. Trial (b) was set out to investigate the effect of an increased sowing rate on the performance of the insecticides. In trial (a) both insecticides were sown in two positions relative to the seed and at three rates. The rates were 1 lb., 2 lbs., and 3 lbs., a.i./acre. A control was also run with these treatments. For the side placement treatment insecticide was sown 1" deep and ½" to one side of a ½" deep seed furrow. Treatments in trial (b) were phorate and Isolan applied at 2lbs. a.i./acre. A control was run also in trial (b). Both insecticide treatments were sown with wheat at 1½ and 3 bushels/acre, while the control was sown with wheat at 1½ bushels/ acre only. An approximation of these sowing rates was 3 seeds per pot for 1½ bushels/acre and 6 seeds per pot for 3 bushels/acre. Five replicates were used in all treatments.

Soil was seived and mixed with perlite with the aid of a concrete mixer. Temperatures within the glasshouse during this experiment fluctuated from 18°C to 27°C. In this experiment pots were sprayed evenly until randomly chosen pots were at approximately 60 % water holding capacity. Plants were infested with aphids every week, two weeks after sowing. The results of this bioassay are recorded in Tables III and IV.

RESULTS.

Table III. The Persistence and Efficiency of Side and Infurrow Placements of Phorate and Isolan indicated by the number of replicates from each treatment showing aphid establishment at intervals after treatment.

Weeks after Treatment			2	3	4	5	6	7	8
Insecticide	Placement	Rate lbs. a.i./acre	No. of Replicates						
Phorate	infurrow	1	0	0	0	2	4	4	5
		2	0	0	0	0	0	0	4
		3	0	0	0	0	0	0	2
	side	1	3	4	2	4	4	5	5
		2	2	2	2	2	3	5	5
		3	2	1	3	3	3	4	5
Isolan	infurrow	1	0	0	0	0	5	5	5
		2	0	0	0	0	3	4	5
		3	0	0	0	0	0	2	5
	side	1	0	1	3	3	4	5	5
		2	0	0	2	2	3	5	5
		3	0	0	0	1	3	4	4
Control			5	5	5	5	5	5	5

Table IV. The effect of Seeding Rates on Persistence of Phorate and Isolan recorded, as the number of replicates that show aphid establishment, at intervals after treatment.

Insecticide	Sowing Rate bushels/acre	Weeks after Treatment						
		2	3	4	5	6	7	8
Phorate (2 lbs. a.i./acre)	1½	0	0	0	0	0	0	2
	3	0	0	1	2	3	3	3
Isolan (2 lbs. a.i./acre)	1½	0	0	0	0	0	2	4
	3	0	0	0	0	2	3	5
Control		5	5	5	5	5	5	5

The results given in Table III are self explanatory. Infurrow applications

of phorate and Isolan gave efficient and persistent control. Phorate sown at the side of the seed, regardless of rates, was not taken up by plants in all replicates and gave inefficient control throughout the experiment. Isolan on the other hand placed in the same position gave excellent control at all rates for at least a month after sowing. With side placement of Isolan there appeared to be no uniform increase in persistence with increased rates of application. The over-all persistence of various application rates in comparison with infurrow applications was markedly reduced.

Trends observed from data presented in Table IV suggest that for phorate, persistence decreases with increased sowing rates, but this did not effect the persistence of Isolan. This difference may be due to phorate's low solubility in water which localizes it, at or near the site of application. With a heavier sowing rate there is a corresponding increase in root density, and it may well be that roots of some plants are screened away from the localized deposits of phorate by roots of other plants in the same replicate. On the other hand Isolan being water soluble, permeates the soil and so may be available to a larger number of plants and area of roots.

EXPERIMENT 4. EFFICIENCY AND PERSISTENCE OF SURFACE APPLICATION TO SOIL.

DESIGN.

This experiment was laid down to investigate the persistence and efficiency of systemic insecticide granules applied to the soil surface.

Preparation of pots and sowing rates of seed were similar in this experiment to those in Experiment 3. Isolan, phorate and a non-treated control were the treatments laid out within this experiment. The three rates at which phorate and Isolan were sown were the same as in the preceding experiment. All rates plus the control treatment had 5 replicates. Plants were treated at the true three leaf stage. Watering was carried out daily with a fine overhead spray. Care was taken not to wash the insecticide from the pots, when watering. Plants were infested with aphids fourteen days after treatment and from then on at weekly intervals.

RESULTS.

Table V shows the results of this experiment.

Table V Efficiency and Persistence of Phorate and Isolan applied at soil surface as indicated by the number of replicates on which aphids establish at 7 day intervals after treatment.

No. of Weeks after Treatment		2	3	4	5	6
Insecticide	Rate lbs.a.i./acre	No. of Replicates				
Phorate	1	4	5	5	5	5
	2	2	5	5	5	5
	3	3	5	5	5	5
Isolan	1	0	0	1	4	5
	2	0	0	0	1	5
	3	0	0	0	0	2
Control		5	5	5	5	5

Phorate gave inefficient control. This was thought to be due to the low water solubility of phorate, which prevented it being washed readily into the root zone. Another factor that may contribute to the poor control given by phorate is its propensity to become bound within the top soil so preventing it reaching the absorptive root zone. Isolan on the other hand gave good control. 1 lb. a.i./acre gave efficient control up to three weeks after treatment. A treatment of 2 lbs. a.i./acre gave control for five weeks after treatment, and 3 lbs. a.i./acre gave control for six weeks. The length of persistency was less than previously shown when Isolan was applied as an infurrow treatment. This difference in persistency is probably due to volatilisation and breakdown by temperature and light, to which the insecticide was exposed when placed on the soil surface.

EXPERIMENT 5. INTERRELATION OF EFFICIENCY AND PHYTOTOXICITY.

DESIGN.

Experiment 5 was laid down following the results of Experiments 3 and 4 within

this section, and of trials carried out on the phytotoxic effects of phorate sown with the seed. An effort was made to try to position phorate away from the seed to avoid phytotoxicity, and at the same time obtain efficient control.

Six replicates were used in each phorate treatment. Phorate was placed in the seed furrow, $\frac{1}{4}$" beneath the seed and mixed uniformly into the top soil immediately surrounding the seed. 2 lbs. a.i./acre were used. A non-treated control consisting of six replicates was added to the experiment and pots were subjected to 18°C. All pots were sown with five plants, and soil was kept at 60% water holding capacity, (see Experiment 1).

RESULTS.

Results showed that the number and rate of germination of the plants, in non-treated pots, did not differ from the treatment in which phorate was mixed throughout the top soil. When phorate was sown in the seed furrow and beneath the seed, germination was observed to be slower and reduced. Both treatments gave efficient initial control. Phorate mixed uniformly throughout the top soil seemed to be the more promising of all treatments, when taking into consideration efficiency of control and phytotoxicity. Efficiency of control was observed by caging aphids on the plant for only 2 weeks after sowing. The experiment was then discontinued.

DISCUSSION OF RESULTS.

Efficient control of R. padi (L.) on barley, wheat and oats was given by phorate and Isolan when applied as furrow applications at the time of sowing. This efficient control of aphids was recorded at rates of 1, 2 and 3 lbs. a.i./acre. With increased rates of application a corresponding increase in the persistence of control was recorded. Phorate applied to wheat and oats at 1 lb. a.i./acre gave efficient control of aphids for six weeks, while 3 lbs. a.i./acre gave efficient control for eight weeks at which time the experiment was abandoned. Isolan at the same rates of application was not as persistent as phorate. The 1 lb. a.i./acre treatment of Isolan was shown to be ineffective at the 4-5 week period, while the 3 lbs. a.i./acre rate gave 7-8 weeks protection, after which time it was recorded as inefficient.

This difference in persistence between phorate and Isolan may be a result of properties of the insecticide and granular base. Granular properties have a direct bearing on the speed with which insecticide is released, (Zaki and Reynolds, 1961). Calcite, in comparison with attapulgite is recognised as a slow release granule because it is more slowly dissolved and broken down in the soil. Attapulgite gives a fast release of insecticide as it disintegrates under moist conditions, but as phorate has a low water solubility and thus a slow and restricted movement through soil, this property is nullified. Conversely Isolan being miscible in water, although based on calcite is quickly dissolved from the granule and permeates the soil. Under constant watering, necessitated by glasshouse conditions, Isolan may be leached out of the root zone area. Reynolds and Metcalf (1962) reported that due to this factor soil applied insecticides with low water solubility gave more persistent control of aphids. Another factor that could have some bearing on the lower persistence of Isolan compared with phorate is the nature of the soil in which the experiment was carried out. Soil used in these experiments was a Templeton silt loam. Getzin and Chapman (1960) observed that Isolan was bound more readily in clayey silt loams than all other soils. It is possible that more Isolan may be adsorbed and bound than phorate in this type of soil.

No clear differences were distinguishable between the persistency of phorate and Isolan under barley, oats and wheat. A trend was observed with barley that persistence of control was shorter with this cereal than with wheat and oats. The difference was not great, and before any conclusion could be drawn, further field and green-house trials would have to be carried out.

Side dressings of phorate were shown to be inefficient regardless of application rate, and this was thought to be due to a slow release and penetration by the insecticide through the soil into the root zone. Isolan on the other hand, with its faster release granule and being miscible with water, gave excellent control although it was not as persistent as infurrow applications.

The persistence of phorate and Isolan was shown to be affected by temperature.

In Experiment 3 it was shown that plants in phorate and Isolan treatments, subject to 24°C, lost their toxicity to aphids more rapidly than similarly treated plants subject to a temperature of 13°C. Treatments of phorate and Isolan at 24°C did not give efficient control after five weeks, while the same treatments at 13°C gave 100% control seven weeks after sowing, after which time the experiment was abandoned.

In treatments subject to 24°C, watering was carried out more frequently in order to maintain the soil at 60% field capacity. This increased rate of watering may have caused leaching of some of the insecticide from the soil which was a free draining mixture of perlite and Templeton silt loam. Other avenues of loss of phorate and Isolan from the soil which are intensified under higher temperatures are:- vapour loss, (Harris et al. 1961), increased uptake from the soil, (Tietz, 1954), and subsequent breakdown (Fukuto, et al. 1961).

An increase in sowing density appeared to give a decrease in persistence of phorate, but no marked difference with Isolan. This may be due to a screening effect given by increased density of plant roots, keeping other roots away from the phorate deposits. The reason for Isolan not exhibiting this, could be due to its more rapid release from the granule, together with its water solubility, facilitating its movement through the soil and so being available to all roots in contrast with a very localised deposit of the insoluble phorate, (Burt et al. 1960).

Applications of phorate and Isolan to the top of the soil followed by daily watering, gave good control from Isolan, but poor control from phorate. Isolan applied in this way gave good initial control but its persistence was of shorter duration than when applied as an infurrow application under similar conditions. This difference is probably due to increased volatilisation from the granules when exposed to the atmosphere. The poor control registered from surface applications of phorate may be a direct result of the toxins slow movement through the soil due to its insolubility in water. A further factor that may effect aphid control from soil surface applications of phorate is the binding of the phorate in the top inch of soil and so not reach the root zone.

Various placements of insecticide in relation to the seed, were carried out to observe if good initial control could be obtained two weeks after emergence without the resulting phytotoxic symptoms of slow and reduced germination. This experiment was carried out with phorate. It was observed that infurrow and beneath the seed applications gave efficient control of aphids at the two week period after sowing, but retarded germination. Insecticide, applied to the side of the seed gave inefficient control of aphids, while phorate mixed into the top soil surrounding the seed gave good control at the two week period without the accompanying phytotoxic symptom. Results on phytotoxic symptoms in Experiment 5 are merely observations. However it does seem plausible that in treatments that have low concentrations of phorate in contact or in the near proximity of the germinating seed would be less detrimental to germination, (see Part III).

P A R T I I

AN INVESTIGATION ON THE CONTACT AND FUME TOXICITY OF PHORATE AND ISOLAN

C H A P T E R 4

REVIEW OF LITERATURE AND METHODS

INTRODUCTION.

An effort was made to evaluate the relative contact and fume toxicities of phorate and Isolan as these insecticides could be readily applied by crop spraying, dusting or topdressing of granules. Such treatments would have the great advantage of alleviating the problem of committing the farmer to aphid control before it was known that such control was necessary in any particular season.

Malathion was used as a basis of comparison for contact toxicity as this substance is a well-known contact aphicide.

REVIEW OF LITERATURE.

No work has been located in the literature on the contact toxicity of Isolan and phorate to aphids. Results of the aphicidal properties of these compounds, recorded from field trials following spraying, reflect the efficiency of two modes of action for these insecticides, namely contact toxicity, and oral toxicity following systemic action. However, results such as these give no indication of the contact toxicity of these materials.

Phorate and Isolan are renowned for their toxicity to mammals resulting from the absorption through the skin. Gordan and Eldefrawi (1960) observed that Isolan was a powerful contact insecticide when applied topically to Musca domestica, Blatella germanica and Oncopeltus fasicatus. Anderson and Atkin (1960) found that bees when subjected to Isolan dust produced high mortalities. Isolan has been shown to be a relatively specific insecticide when applied in sprays. van de Vrie (1963) reported that Isolan had no direct effect on predatory Typhlodromid mites. Ankersmit, Locher, Vethuis and Zwart (1963) also found that hymenopterous parasites and predators were not effected to any great extent by Isolan sprays. Isolan has been observed to be a more efficient contact than a systemic aphicide. Good aphid control has been recorded at lower concentrations than when systemic activity was detected, (Anon 1962).

Phorate has also been reported to be a powerful contact insecticide. Areekul and Harwood (1962), observed that phorate was toxic when topically applied to various insects used in bioassay work. Metcalf and Fukuto (1960) found phorate was toxic to Tetranychus telarius, Musca domestica and other insects when topically applied to their cuticle. Phorate's contact toxicity to Musca domestica was substantiated by Bowman and Casida, (1959).

Both phorate and Isolan have been shown to emit fumes that are toxic to Aphis fabae, (Anon, 1961). Etheridge (1961) substantiated this property of Isolan in tests with other species of aphids. Cook, (1959), observed that phorate granules control the pea aphid on lucern when it is applied topically to the plant. This author reported that control was effected by the toxic fumes emitted from the phorate granules. Similar claims have been made by Mulholland, (pers.com. 1963) who controlled the cereal aphid on wheat with aerial applications of phorate granules. Schroeder (pers.com. 1963) and Hall (pers.com. 1964) also reported aphid control from fumes emitted by phorate following field application of granules. Lindley (1962) also found that topical applications of phorate granules gave efficient control of Myzus persicae. Reynolds, Fukuto and Peterson (1960) claimed excellent control of Myzus persicae on sugar beet following topical applications of phorate granules. Analysis of the treated vegetation led these authors to the opinion that control resulting from phorate applied in this manner was due to the systemic and fumigant properties of this toxicant, while its contact action played little or no part in the overall control obtained. However, other authors are of the opinion that topdressing of granules depends on contact and fume toxicity of phorate rather than its systemic effect for control.

METHODS.

Contact Toxicity.

The topical application technique used was developed by Harrison (1961). Application of this technique required the construction of a self-filling micro-pipette. This consisted of a capillary tube fixed and sealed into a holding tube to which compressed air was introduced to eject the solution from the capillary tube.

The capillary tube was self-filling due to capillary action. Construction of this fine measure was achieved by drawing out molten "Pyrex" tubing to the required diameter. The diameter was measured under a binocular microscope after which the necessary length was cut. Lengthwise measurement of the tube was accomplished with a micrometer screw gauge.

The length of capillary tube of known diameter was cut so that the volume was approximately 1/40th of the test organisms body weight. The average weight of a mature apterous aphid was 6.4 mg.

Various solvents in which to dissolve the three insecticides were screened for the following properties:

(a) non-toxic to aphids;
(b) would dissolve phorate, Isolan and malathion;
(c) easy to apply to the test insects;
(d) possesses a sufficiently low vapour pressure to avoid losses by evaporation when testing.

Kerosene was of little use as it would not dissolve malathion or Isolan. In comparison clove oil had all the properties of the required solvent but was very messy, and aphids in control treatments died from being stuck to the walls of the post treatment cages. Added disadvantages were that a residual amount of oil could not be expelled from the needle, and the oil would not spread evenly onto the cuticle of the aphid. Methyl cyanide and xylol were found to be very suitable solvents but under high temperatures evaporated quickly. Xylol was eventually used, and provided testing was carried out on cool days or in the cool of the evening and the operation took not more than 12 to 15 seconds to treat each individual, then losses from evaporation were negligible.

Xylol when applied to the cuticle diffused so rapidly through the cuticle wax that no drying period was needed before placing aphids into the recovery container.

Test solutions were made up initially on a weight/volume basis, and subsequently diluted to the required concentration. Ranges of concentrations of test solutions were estimated by small trial and error tests. Solutions were fed into the capillary needle by means of a wire loop. A film of solution was formed

within the loop by dipping into the test solutions. When this was brought in contact with the needle it was drawn through the needle by capillary action.

Insects for testing were held in place by a suction tube. It was shown that if the holding tube was made from thick walled glass and ground flat at the suction end, the occurrence of cuticle rupture of aphids was reduced. Aphids were picked up by the dorsal side of the abdomen by this piece of apparatus and treated on the ventral side of the abdomen. After treatment the aphids were dropped into a container by cutting off the suction pressure to the holding tube.

Aphids were placed for a 24 hour post treatment period in 'Clippa' plastic containers fitted with clamp-on lids. The centre of these soft plastic lids was cut out and nylon mesh placed on the inside of the lid when sealing the container, thus leaving air access through the lid and nylon mesh. Plant material on which the aphids could feed during the recovery period was also enclosed. Containers were then placed in a cabinet set at 18°C and 90% relative humidity.

Fume Toxicity.

An endeavour was made to assess the fumigant toxicity of both phorate and Isolan. However there appears to be no solvent in which all these compounds could be diluted which did not have vapour, toxic to the aphid. To get over this difficulty a known weight of insecticide was dissolved in chloroform and made up to known concentrations. 0.25 ml of these insecticide solutions were then run out onto filter paper. The filter paper was rapidly dried under a cool fan and placed in a 250 ml flask, leaving a residue of insecticide. Into the top of this flask was fitted a quick-fit joint with a 6 inch length of 2 inch diameter glass tube. A cintered glass partition was placed 4 inches up this tube and the end of the tube capped.

Aphids for testing were dropped through the neck of the tube onto the cintered glass plate. The flask was sealed by replacing the ground glass cap.

Twenty aphids were used in each flask. Flasks after treatment were placed in a cabinet at 18°C and left for 12 hours.

A control was incorporated into each test to which chloroform had been added and evaporated from the paper filter.

CHAPTER 5

RESULTS AND DISCUSSION

CONTACT TOXICITY.

The number of deaths for each concentration of insecticide was assessed and the percentage deaths corrected by Abbott's formula .

This data was then transformed, and the percentage mortality based on a probit scale and the percentage concentration on a log scale. With this transformation a straight line regression was calculated, according to Busvine (1957). Data was checked with the X^2 test for homogenity, and 95% confidence levels were estimated at the LD50 and LD95. These two mortality levels were chosen as an index of comparison of the three insecticides.

Tables I and II give the results of observed data and important determinations after analysis. Log/probit regression lines for each insecticide are shown in Figure 3.

Table I Results of Topical Application of Xylol solutions of Isolan, Phorate, and Malathion to Adult Apterae of Rhopalosiphum padi (L.)

	Malathion				Phorate				Isolan			
Test No.	No. of Insects	% Conc.	% Mort.	Correct % Mort.	No.of Insects	% Conc.	% Mort.	Correct % Mort.	No.of Insects	% Conc.	% Mort.	Correct % Mort.
1	30	0.08	90	89.6	30	0.06	93.3	93.3	30	0.008	96.6	96.35
2	30	0.06	82.1	81.0	30	0.040	83.3	83.3	30	0.006	90.0	89.20
3	28	0.04	64.3	63.0	30	0.020	63.3	63.3	30	0.004	80.0	78.58
4	28	0.02	75*	73.24	30	0.006	13.3	13.3	30	0.002	63.3	56.1
5	30	0.01	11.4	8.33	30	0.004	6.6	6.6	30	0.0008	20	14.34
6	30	0	3.33		30	0	0		30	0	6.6	

*Note: Holding cages were suspected of being contaminated. Results were therefore not used in analysis of data.

FIGURE 3

Log/probit regression lines showing the relative contact toxicities of malathion, phorate and Isolan

PROBIT MORTALITY LOG CONCENTRATION REGRESSION LINES

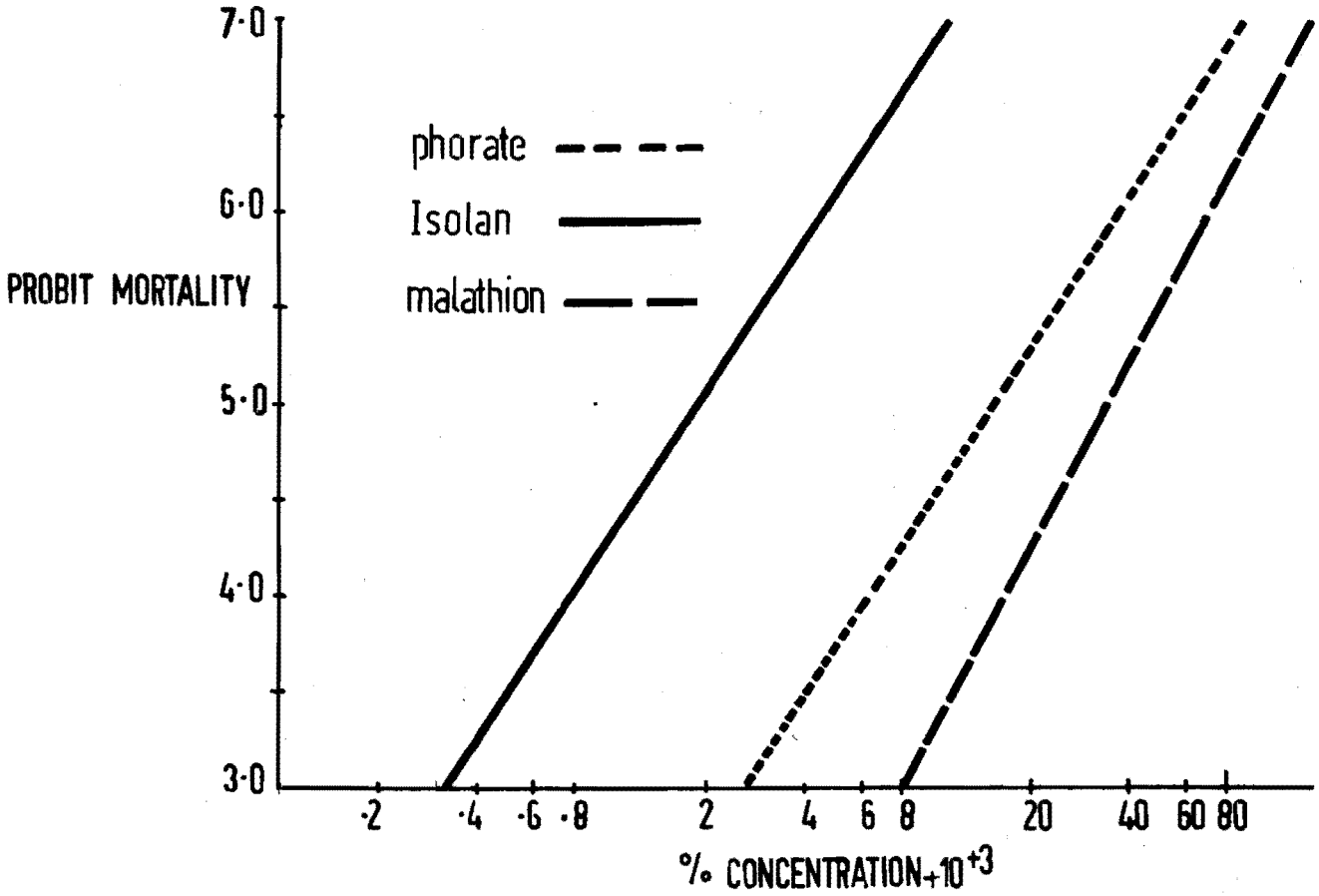


Table II Results of Analysis of Data from Table I giving a Comparison of Toxicities of Three Insecticides at LD50 and LD95.

Insecticide	ug/gram of Aphid at LD50	95% Confidence Limits	ug/gram of Aphid at LD95	95% Confidence Limits	Regression Coefficient	Comparison with Malathion		Comparison with Phorate	
						LD50	LD95	LD50	LD95
Malathion	0.890	+ 0.141 - 0.120	3.294	+ 1.282 - 1.340	2.888	-	-	-	-
Phorate	0.394	+ 0.069 - 0.065	1.728	+ 0.592 - 0.442	2.538	2.25	1.90	-	-
Isolan	0.0486	+ 0.0068 - 0.0089	.1955	+ 0.0569 - 0.0740	2.624	18.31	16.84	8.11	8.86

The results of LD50 and LD95 are expressed as ug. of insecticide per gram weight of aphids. One gram of aphid represents approximately 154 mature apterae. When logarithm doses are converted to actual doses the 95% confidence range is off-centre in respect to the LD levels, i.e. LD50 and LD95, (see Table II).

FUME TOXICITY.

Fumes arising from .25 ml of 0.1% and 0.01% of phorate and Isolan solutions at 18°C. over a period of twelve hours gave 100% kill of aphids, while the controls registered no kills.

DISCUSSION.

It was evident when comparing malathion and phorate at the LD50 level that phorate under these laboratory conditions, was at least 2.25 times as toxic as malathion. At the LD95 level phorate was 1.90 times as toxic as malathion. However comparing the two at the LD95 level it was shown that the lower confidence limit of malathion merges with the higher limit of phorate. Isolan when compared with malathion at the LD50 level was 18.31 times as toxic, and at the LD95 level it was 16.84 times as toxic. Isolan when compared with phorate was 8.11 times as effective at the LD50 and 8.86 at the LD95.

For field assessment from laboratory data the LD95 level of comparison is more valid as it is nearer the range for a 100% control at which most field applications are aimed. This laboratory assessment of contact toxicity gives only

a pointer to the relative values of various insecticides, and is only of use for screening insecticides for use in the field. Under field conditions other chemical properties have a large bearing on the performance of insecticides in relation to control. Both phorate and Isolan are very volatile and Isolan is water soluble, hence the superiority exhibited by Isolan as contact aphicides, over the other two insecticides in the laboratory may not be substantiated in the field.

Fumes of both phorate and Isolan are very toxic as shown by 100% deaths of aphids subjected to fumes of 0.25 ml of 0.1% and 0.01% of phorate and Isolan solutions. In the field excellent control has been recorded for phorate fumes, (Mulholland pers.com. 1964, and Schroeder pers.com. 1964).

As technical products of phorate and Isolan are very efficient contact and fumigant aphicides under laboratory conditions it must be concluded that as foliar applications of granules, dusts or sprays both materials should perform creditably, under field conditions.

P A R T I I I

THE PHYTOTOXICITY

OF

PHORATE AND ISOLAN GRANULES

APPLIED TO THE SOIL

C H A P T E R 6

REVIEW OF LITERATURE AND METHODS

INTRODUCTION.

This section was devoted to investigating the phytotoxic effects of phorate and Isolan as granulated soil applied systemics. Compatibility with plant growth is a prime requisite for all systemic insecticides.

REVIEW OF LITERATURE.

With the discovery of stable and efficient contact and systemic insecticides developed the practice of soil and seed insecticide applications. However some insecticides under certain conditions were shown to be phytotoxic to the plant. The degree of phytotoxicity appears to depend on:-

(a) The species of plant being treated.
(b) The insecticide used and the formulation of it.
(c) The environmental conditions under which the insecticide is applied.

Some plants are more prone to damage than others. Lange et al. (1949) showed that some species of beans were more susceptible to damage by crude BHC than other plants. Hall (1951) observed that carnations and tomatoes developed a number of abnormalities when subjected to sprays of TEPP and HEPP. This claim was substantiated by Brook and Anderson (1947). Reynolds et al. (1957) observed that sorgum seed when coated with phorate, Systox and Disyston gave a very poor stand when compared with cotton, alfalfa and sugar beet, while the emergence of soya beans from coated seed was almost a complete failure.

Bardiner (1964) showed that prior and during germination phorate can enter the developing embryo of wheat seed, while mustard seed possesses a testa impermeable to phorate. This may be one reason why certain species of seeds are tolerant to the phytotoxic effects of insecticides.

Symptoms and severity of damage vary with the chemical used. Kostoff (1948) working with various graminaceous plants showed that BHC caused a depression in stem and root growth due to abnormal miotic divisions resulting in polyploidy.

Zimmerman et al. (1947) reported that TEPP and HEPP induced epinasty in over twenty species that were treated. With tomatoes and carnations Hall (1951) recorded that treatment by TEPP and HEPP produced epinasty, very branched flower branches, thick leathery sepals and prominent ovaries containing mature seeds, while the rest of the plant was still vegetative. Reduction in germination resulting from granular or seed applications of Disyston and phorate was observed with wheat by Bardiner (1960), Brown (1957, 1960); and on cotton by Parencia et al. (1957), Reynolds et al. (1957), Zaki and Reynolds (1961), Leigh (1963), and Hacskaylo and Ranney (1961). Stunted growth and retarded germination in wheat were recorded by Brown (1960). Wallace (1962), Leigh (1963), Harwood and Bruehl (1961) and Hacskaylo et al. (1961) working with cotton seed treated with phorate observed a retarded effect on germination. Marginal burning of cotton and sugar beet arising from seed treatments or granular applications of phorate has been observed (Zaki and Reynolds 1961, Parencia et al. 1957, Ripper 1957, and Anon 1961).

Wallace (1960) showed that germination of subterranean clover was suppressed by Metasystox and Sayfos, the former having the greater effect. Slight stunting and loss of colour were evident in the seedlings but these recovered quickly. Phorate was relatively non-phytotoxic to subterranean clover.

Arneson et al. (1947) showed BHC treated potato tubers gave a poor sprouting response. Systox was also observed to affect the sprouting response and gave leaf burning when applied as a seed soak to tubers (Klostermeyer 1953). Poor germination was recorded from potato tubers treated with dust and granules of phorate (Pond 1963, Pigotti and Orlando 1962). Burt et al. (1960) have encountered poor growth rates with seed potatoes saved from Disyston and phorate treated plants.

The various formulations of insecticides have been shown to exhibit varying propensities to introduce phytotoxicity. Hocking (1949) proved that the malformations induced by crude BHC were caused by trichloro benzene metabolites of the alpha isomer. Hence when only the gamma isomer of lindane was used this damage was reduced. Dimethoate was shown to be more phytotoxic than phorate or Systox. Ripper (1957) claims that in general phorate does not appear to be as phytotoxic as Disyston.

Bardiner (1960) considers that the two most important factors influencing the phytotoxicity arising from seed coat applications are :-

(a) Concentrations of the active ingredient adhering to the seed.

(b) Variations in the release of insecticides from the formulated material

The variations under (b) can be subdivided into :-

(i) Differences in the amount of insecticide penetrating the seed coat before germination.

(ii) Variations in the speed at which the insecticide becomes available for absorption by the plant or seed.

By using slow release stickers such as P.V.A. and Aroclor for seed coat treatments, Bardiner (1960) demonstrated that phorate and carbon when applied to wheat seed reduced the phytotoxicity and increased persistence. Zaki and Reynolds (1961), working with phorate, Systox, and dimethoate granules formulated on vermiculite, showed these to be more phytotoxic than when formulated on attaclay. The former gives a much faster release of insecticide. Brown (1960) observed that phorate granules applied at more than 1 lb a.i./acre tended to increase phytotoxicity producing a more stunted plant.

Seed treatment is in general more toxic than granular applications (Ripper 1957, Brown 1960, and Zaki and Reynolds 1961). Reynolds et al. (1957) found that dimethoate applied as an emulsion was more phytotoxic than seed treatment or granular applications.

The enviromental conditions under which the insecticide is applied to the soil is of paramount importance in the occurrence of phytotoxicity. Ripper (1957) reports that temperature and moisture influence germination of treated cotton. Under conditions that were near optimum for cotton germination (75° to 80°F at 30 % field capacity) the reduction resulting from seed treatment was negligible. However under more adverse conditions (60 to 65°F at 60-70 % field capacity) phorate seed treatment caused a large reduction in germination (Ripper 1957). Haeskaylo and Ranney (1961) observed that cotton seed germination was higher with moisture levels

of 50% of field capacity than with 75%. Cotton stands from phorate treated seed, germinated at 77° to 86°F. and at 55% field capacity showed no reduction in germination. Phorate treated seed showed a marked reduction in the rate and percentage of germination when cotton was sprouted at 67° to 72°F. at 50% field capacity; this reduction was aggravated still further by an increase in soil moisture to 70% water holding capacity. Reduction of germination under these conditions may increase with use of inferior seed.

The apparent phytotoxicity of BHC was found to be reduced by the addition of a fungicide to the seed with seed treatment. Adkisson (1958) working with cotton observed that if the seed was treated with nanbam before soil or seed treatment with Disyston or phorate a better germination resulted. Allen et al. (1963) found that sugar beet seed treated with diazinon or phorate showed reduced germination, but that with the addition of captan a better stand resulted. Erwin, Reynolds, and Garber (1961) reported phorate or a product resulting from its degradation predisposes the cotton seed to attack by a pathogen of Pythium species which reduces emergence.

Soil factors have been shown to be very important in ascertaining whether a given dose of insecticide when applied to the seed or soil will be toxic to the plant. Disyston and phorate gave varying phytotoxic symptoms when applied to differing soil types. The severity of damage in relation to soil type followed in decreasing order: sandy loam > loamy fine sand > silty loam >clay loam, (Zaki and Reynolds 1961). This order is very similar to that observed by Casida et al. (1952) for the amount of insecticide taken up from various soils. This gradation of phytotoxicity resulting from applications made to various types of soil, is probably a result of the amount of insecticide which is released and is unattached to the soil. With light sandy soils less of the released insecticide will be bound, as the quantity of soil colloids on which the insecticide may be adsorbed is less than with heavy texture soils, and thus more is taken up by the plant.

Various practices have been employed to lessen phytotoxicity of different chemicals. Of these practices the placement of the insecticide in relation to the

position of the seed, is the most important. The insecticide is not brought in contact with the plant until after germination when the seed is less prone to damage. As the roots spread, the insecticide should be in such a position that the roots come in contact with it and absorb it. Reynolds et al. (1957) showed that with sugar beet, cotton and alfalfa the spraying of an emulsion on the soil prior to sowing gave a good control and a low incidence of phytotoxicity as compared with results when the emulsion was sprayed on the seed at the time of sowing. Similar results were found with granules broadcast over the soil prior to planting c.f. furrow treatment. Harding and Wolfenbargen (1963) outlined three ways of applying systemics, all of which gave good control:

(a) Infurrow beneath or next to the germinating seed.
(b) Side-dress application after the plant has emerged.
(c) Soil or surface application at the time of planting or prior to irrigation.

Zaki and Reynolds (1961) showed that phorate and Disyston applied ten days after planting of cotton seeds had no effect on germination compared with in seed furrow application, but gave efficient control of insects.

Excellent results have been achieved in insect control by sowing either below or at the side of the seed. Brown (1960) considers that the nearer the phorate granules are placed to the seed the better the control. Tsai and You (1962) observed, when sowing phorate granules in three separate positions relative to the seed: immediately below the seed, 1 cm. under the seed, and 3 cm. to one side of the cotton seed, that germination was reduced in the seeds in the first two positions.

Growth stimulation with phorate has been reported by Brown (1957) and (1960) who showed that treating wheat for hessian fly control gave an increase in plant growth. This increase could not be attributed to hessian fly control as the increase occurred with the resistant variety Ponca. Wallace (1960) when treating Dwalgemup subterranean clover with Systox and Metasystox to seeds for control of Smynthrus viridus noted, that after three months there was a 39% and 24% increase respectively in plant weight. Smaller differences were shown for phorate and Sayfos treated seeds grown outside. Wallace (1961) is of the opinion that this phenomenon

is tied up with an influence on the rhizosphere. Zaki and Reynolds (1961) noted an increase in the growth rate of cotton, treated with Disyston and phorate. This may be an effect on the micro-organisms of the soil. Hacskaylo and Stewart (1957) recorded that cotton seed treated with phorate was not effected by Rhizoctonia solani. Erwin, Reynolds and Garber (1959) reported that in soil infested with R. solani, phorate increased cotton stands. Hacskaylo and Stewart (1962) showed that phorate treatment of cotton controlled Rhizoctonia within the temperature range of 82°-92°F. but not within the range of 70°-72°F. Phorate was not toxic to Pythium debaryanum and Fusarium maniliforme. Erwin et al. (1961) observed that phorate predisposes cotton to attack by Pythium species. Therefore at the right temperature and in the absence of Pythium species, phorate could be beneficial to cotton germination, especially in the presence of R. solani.

METHODS.

All designs of experiments were influenced by a large pilot trial which was set out in two growth cabinets. The design of this pilot trial was a two, by two, by three. Pots within the experiment were subjected to two temperatures, 22°C and 13°C. The treatments under these temperatures were: soil type, moisture level, and insecticidal treatments. These factors were chosen because various authors had shown that temperature, moisture level, and soil type, influence the phytotoxic effects of phorate to a marked degree (Hacskaylo and Ranney 1961 and Reynolds et al. 1962).

All seed stocks used had a 98% germination test from which only well formed seeds were used in trials. Following information gained from this preliminary experiment, all further experiments on plant growth were carried out in soil at approximately 60% field capacity. The technique for determining soil moisture is outlined in Part I. Intervals between watering varied with environmental conditions.

Dry weights of plant material from plants harvested at soil level were obtained as follows: The leaves and stems were separated when green and the total placed in brown paper bags. These bags were then placed in an oven set at 27°C. and left for 24 hours, then removed and placed in a dessicator to cool. After this

treatment the plants were removed and weighed. Total dry weights of the plants within each replicate, together with the leaf and stem weight, were recorded. From these figures the stem to leaf dry weight ratios were determined.

Observations on germination were made every day. Prior to analysis, all germination recordings were converted to a percentage of the total number of seeds sown and converted to $\sqrt{\text{arc sine}}$ %. This transformation was made in order to correct readings for binomial distribution.

C H A P T E R 7

EXPERIMENTAL DESIGN RESULTS AND DISCUSSION

EXPERIMENT 1. INHIBITION OF GERMINATION EXHIBITED BY INFURROW APPLICATIONS OF PHORATE AND ISOLAN.

DESIGN.

This trial was laid down initially to observe the persistence and efficiency of soil applied insecticides phorate and Isolan treated to wheat, barley and oats. However, observations made from day to day on the emergence of seedlings after being sown with phorate and Isolan at 1, 2 and 3 lbs. a.i./acre gave some very enlightening data, with respect to the phytotoxicity of these materials.

After emergence counts were taken, 3 replicates in each treatment were eliminated from the trial. The remaining 5 replicates were used to determine the efficiency, and persistence of phorate and Isolan for aphid control (Part I, Experiment 1) and to observe the effect of these insecticides on plant growth.

Three well formed seeds were selected for sowing in each pot. The glasshouse temperature was set at 15.5°C but temperatures fluctuated from 14°C to 18°C

throughout the experiment. Emergence was recorded 7 days after seeds in the control had emerged.

RESULTS.

Results of this experiment are shown in Table I and the analysis of this data is given in the Appendix II. This procedure is followed throughout Part III.

Table I The Percentage Germination recorded 7 days after Emergence was first observed in the Controls.

Treatment	Rate lbs. a.i./acre	Wheat	Barley	Oats
Control	0	100	100	100
phorate infurrow application	1	95.83	87.50	100
	2	87.50	75	95.83
	3	66.66	58.33	100
Isolan infurrow application	1	100	100	100
	2	95.83	95.83	100
	3	95.83	95.83	100

Prior to analysis all percentages were transformed to $\sqrt{\text{arc sine } \%}$

Comments resulting from analysis of data (Table I).

(1) All the results in Table I were analysed for variance. Analysis showed that only trends observed with barley were significant. Phorate gave a significant reduction in germination in comparison with Isolan, ($F = 19.06^{*}$). Although this trend was not significant for wheat it was nevertheless present. In comparison oats seemed to be tolerant to the phytotoxic properties of phorate.

(2) Phorate was observed to retard the germination of wheat and barley and this phenomenon is shown on barley in Plate 2.

* = significant at the 5% level.

** = significant at the 1% level.

(3) Nine weeks after sowing, wheat plants treated with phorate were observed to be severely stunted (Plate 3).

EXPERIMENT 2 RETARDATION AND INHIBITION OF GERMINATION CAUSED BY PHORATE AND ISOLAN

DESIGN

The aims of this experiment were to demonstrate the retardation and suppression of wheat emergence induced by phorate and Isolan, and to study these effects under varying rates at differing sites of application.

The experiment was based on 130 pots. Each pot was sown with 5 selected seeds. Phorate and Isolan were applied at two positions relative to the seed and at three rates of application, 1,2, and 3 lbs a.i./acre. Each rate of application contained ten replicates and in addition a control was added consisting also of ten replicates. Observations of emergence were made daily for six days after the first control treatment plants emerged. For analysis this six day period was divided into two phases. The first phase was taken as the first two days and the second phase as the three to six day period.

Glasshouse temperatures during the experiment fluctuated between 15.5°C and 23°C.

RESULTS

Table II Number of plants emerging in the 1st and 2nd phase are shown as a percentage of the seeds which emerged.

RATE		1 lb a.i./acre		2 lbs a.i./acre		3 lbs a.i./acre	
Insecticide	Placement	1-2 day	3-6 day	1-2 day	3-6 day	1-2 day	3-6 day
phorate	Infurrow treatment	18.1	81.9	12.7	87.3	15.5	84.5
	Side treatment	75.0	25.0	80.0	20.0	77.5	22.5
Isolan	Infurrow treatment	50.0	50.0	43.2	56.8	35.5	64.5
	Side treatment	86	14.0	79.6	20.4	68.7	31.3
Control		84	16.0				

Results for the 1st phase were analysed after being transformed to $\sqrt{\text{arc sine}}$ %.

Comments resulting from analysis of data (Table II).

(1) The coefficient of variance (C.V.) was calculated 9.34%.

(2) Phorate suppressed emergence more severely than Isolan, ($F = 12.46^{*}$).

(3) Infurrow applications of phorate and Isolan had a much greater detrimental effect on germination than the side placement applications of these insecticides, ($F = 132.16^{**}$).

(4) An interaction occurred between placement and insecticide, ($F = 10.72^{*}$). To further illucidate this interaction the means of the placement and type of insecticide were compared by using Duncans Multiple Range Test. These transformed % means are listed below:

		1%	5%
Isolan side treatment	62.37%	A	a
Phorate side treatment	61.70%	A	a
Isolan infurrow treatment	40.86%	B	b
Phorate infurrow treatment	23.06%	C	c

Duncan's test showed that both at the 1% and the 5% level there was no difference in significant trends. Results from side treatments of phorate and Isolan did not differ significantly from one another, but there was a marked difference when results were compared with those from infurrow treatments of phorate and Isolan. Phorate infurrow treatment was shown to retard germination to a greater extent than Isolan.

These means were compared with the control by the T test. The control did not differ from the two side treatments but was significantly different (1% level) from infurrow applications of phorate and Isolan.

On the 6th day the total number of seeds germinated were taken as a percentage of the number planted and transformed to $\sqrt{\text{arc sine}}$ %. These figures were then analysed as treatment totals rather than as individual replicates.

Table III Number of Wheat Seeds in each Replicate six days after Emergence Commenced.

Insecticide	Placement	Rate lbs. a.i./acre	Number of Replicates									
Phorate	Infurrow	1	5	5	4	5	5	4	5	5	5	4
		2	5	4	3	5	5	5	3	4	5	4
		3	4	3	5	3	2	3	4	4	5	5
Phorate	Side	1	5	5	5	3	5	5	5	5	5	5
		2	5	5	5	5	5	5	5	5	5	5
		3	5	4	5	5	5	5	5	5	5	5
Isolan	Infurrow	1	5	4	5	5	5	5	5	5	4	5
		2	5	3	5	4	3	5	5	4	5	5
		3	5	4	5	5	5	3	4	4	5	5
Isolan	Side	1	5	5	5	5	5	5	5	5	5	5
		2	5	5	4	5	5	5	5	5	5	5
		3	5	4	5	5	5	4	5	5	5	5
Control			5	5	5	5	5	4	5	5	5	5

Each replicate was transformed to $\sqrt{\text{arc sine}\%}$ and the sums of each treatment analysed.

Comments resulting from analysis of data Table III.

(1) The C.V. was found to be 5.1%.

(2) Phorate and Isolan did not differ significantly in their effect on germination.

(3) Infurrow applications of phorate and Isolan were significantly more detrimental to emergence and germination than side applications, ($F = 19.7^{**}$).

EXPERIMENT 3. EFFECT OF PHORATE AND ISOLAN ON GROWTH OF WHEAT.

DESIGN.

The aim of this experiment was to assess the effect of phorate and Isolan on

plant growth when applied to the soil in various positions. This experiment is a continuation of Experiment 2. The whole experiment consisted of 260 pots. Within these 260 pots were the 130 pots on which observations for Experiment 2 were based. Treatments were similar to Experiment 2 but instead of having 10 replicates for each rate of application, there were 20. All pots were sown with 5 well formed seeds. After emergence seedlings were thinned so that only three plants per pot were allowed to develop. This practice was an insurance that replicates had equal numbers of plants. For higher infurrow application rates of phorate and Isolan, two more replicates per harvest were sown as lower germination was anticipated. Five replicates for each treatment were harvested two weeks after sowing, and harvests were carried out fortnightly for a period of eight weeks.

Fluctuations of temperature between 15.5°C and 26°C were recorded in the glasshouse during the time the experiment was run. Watering was carried out daily as the soil tended to dry out under glasshouse conditions.

RESULTS.

Table IV A Comparison of Dryweights (grams) of Wheat Plants, harvested at 14 days after sowing in soil treated with phorate and Isolan.

Treatments			Replicate Nos				
Insecticide	Placement	Rate lbs.a.i./acre	1	2	3	4	5
Phorate	infurrow	1 lb.	.044	.051	.042	.058	.056
		2 lb.	.045	.032	.025	.031	.040
		3 lb.	.038	.035	.030	.050	.041
Phorate	side	1 lb.	.061	.077	.078	.054	.073
		2 lb.	.070	.054	.049	.066	.062
		3 lb.	.061	.057	.064	.067	.076
Isolan	infurrow	1 lb.	.057	.070	.057	.048	.040
		2 lb.	.064	.063	.059	.056	.042
		3 lb.	.031	.056	.066	.056	.056
Isolan	side	1 lb.	.064	.060	.071	.067	.062
		2 lb.	.060	.074	.058	.064	.061
		3 lb.	.057	.058	.074	.062	.048
Control			.070	.067	.071	.072	.067

PLATE 2

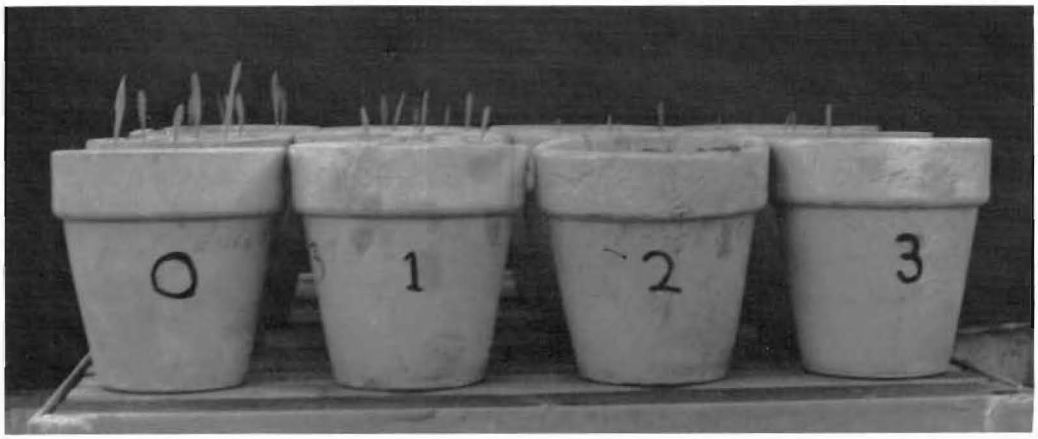
The retarding effect of phorate on the germination of barley.

Left to right - control, phorate 1 lb. a.i./acre.
phorate 2 lbs. a.i./acre, phorate 3 lbs. a.i./acre.

PLATE 3

The stunting effect of phorate on wheat.

Left to right - control, phorate 1 lb. a.i./acre,
phorate 2 lbs. a.i./acre, phorate 3 lbs. a.i./acre.


0
1
2
3


67
87
17
P

Comments resulting from analysis of data (Table IV).

(1) The standard error (S.E.) was calculated (0.008 grams) and the C.V. was found to be 14.9%.

(2) Phorate was significantly more deleterious than Isolan, ($F = 7.08^{**}$).

(3) Infurrow applications of phorate and Isolan affected plant growth much more severely than side applications of the same insecticide, at the two week stage, ($F = 50.77^{**}$).

(4) There was no significant differences between 1 lb. rates of application and the 3 lb. rates of application.

(5) An interaction was shown to be highly significant between insecticide and placement, ($F = 12.3^{**}$). The means of the treatments constituting this interaction were compared with a Duncan's Test, and then compared with the control.

		1%	5%
Phorate side treatment	.064	A	a
Isolan side treatment	.063	A	a
Isolan infurrow treatment	.055	A	b
Phorate infurrow treatment	.041	B	c

At the 5% level side placement of phorate and Isolan gave similar results. The plants from these treatments were heavier than those treated with phorate and Isolan in the seed furrow. At the 1% level Isolan infurrow application, unlike phorate, was not significantly different from side application of both insecticides.

These treatment means were compared with the control and it was observed that the control was not significantly different from both phorate and Isolan side applications, but was significantly different from Isolan infurrow application at the 5% level and phorate infurrow application at the 1% level.

RESULTS.

Table V A Comparison of Dryweights (grams) of Wheat Plants, harvested 28 days after sowing in soil treated with phorate and Isolan.

Treatments			No. of Replicates				
Insecticides	Placement	Rate lbs.a.i./acre	1	2	3	4	5
Phorate	infurrow	1 lb.	.284	.424	.329	.431	.225
		2 lb.	.484	.447	.496	.496	.500
		3 lb.	.327	.279	.328	.194	.295
Phorate	side	1 lb.	.379	.373	.405	.372	.405
		2 lb.	.392	.392	.423	.343	.302
		3 lb.	.398	.401	.386	.423	.448
Isolan	infurrow	1 lb.	.408	.482	.358	.452	.379
		2 lb.	.433	.366	.335	.381	.290
		3 lb.	.423	.432	.385	.381	.280
Isolan	side	1 lb.	.456	.320	.413	.335	.349
		2 lb.	.325	.309	.459	.356	.315
		3 lb.	.360	.404	.365	.400	.459
Control			.360	.444	.396	.424	.392

The numbers in Table V were converted to whole numbers and analysed for variance.

Comments resulting from analysis of data (Table V).

(1) The S.E. was calculated to be 0.054 grams and the C.V. 14.77%.

(2) Phorate was found to be significantly more deleterious to plant growth than Isolan, ($F = 8.50^{*}$).

(3) Infurrow application of phorate and Isolan was more damaging to plant growth than side application of these insecticides, ($F = 10.10^{**}$).

(4) An interaction between the two insecticides and placement was highly significant ($F = 15.70^{**}$). This interaction was further analysed with a Duncan's Test. The means constituting the interaction were then compared with the control.

		1%	5%
Phorate side treatment	0.389 grams	A	a
Isolan infurrow treatment	0.386 grams	A	a
Isolan side treatment	0.378 grams	A	a
Phorate infurrow treatment	0.290 grams	B	b

At the 1% and 5% level the only treatment that differed significantly from the other three treatments was the infurrow application of phorate

When the above means were compared with control it was found that phorate infurrow treatment was the only treatment to differ significantly, (1% level).

The third harvest which was carried out at the six week period after germination was analysed in the same way as the previous two harvests.

RESULTS.

Table VI A Comparison of Dryweights (grams) of Wheat Plants harvested 42 days after sowing in soil treated with phorate and Isolan.

Treatments			No. of Replicates				
Insecticide	Placement	Rate lbs.a.i./acre	1	2	3	4	5
Phorate	Infurrow	1 lb.	1.146	1.079	1.071	1.230	1.105
		2 lb.	1.166	1.253	1.239	1.295	1.239
		3 lb.	.955	1.270	.620	.921	1.443
Phorate	Side	1 lb.	1.188	1.045	.990	1.287	1.378
		2 lb.	1.173	1.125	1.160	1.270	1.151
		3 lb.	1.270	2.050	1.370	1.183	.943
Isolan	Infurrow	1 lb.	1.303	1.248	1.328	1.466	1.262
		2 lb.	1.447	1.330	1.449	1.514	1.402
		3 lb.	1.280	1.481	1.326	1.283	1.615
Isolan	Side	1 lb.	1.298	1.930	1.603	1.378	1.675
		2 lb.	1.770	1.503	1.110	1.154	1.513
		3 lb.	1.443	1.300	1.503	1.302	1.497
Control			1.323	1.608	1.899	1.570	1.890

Comments resulting from analysis of data (Table VI).

(1) The S.E. was calculated (0.210 grams) and the C.V. was found to be 15.8%.

(2) Phorate was more deleterious to plant growth at the six week period than Isolan (19.7**).

(3) There was no significant difference between side and infurrow applications of insecticides.

(4) No significant rate differences or interaction between insecticide and placement emerged from this data.

(5) When the treatment means were compared with the control using a T test, it was found that Isolan side treatment did not differ significantly from control. However Isolan infurrow treatment differed from the control at the 5% level while both phorate treatments differed at the 1% level.

(6) Tiller numbers, leaf numbers, and leaf to stem dry weight ratios were recorded in order to observe if the difference in total dry weights in phorate and Isolan infurrow application treatments were due to a uniform stunting effect on the plants rather than a morphological abnormality. Data are recorded in Appendices VIII, IX, X and XI. Analysis of these data showed no significant differences between treatments, suggesting that the reduction in dry weights of insecticide treated wheat plants were due to a uniform stunting.

RESULTS.

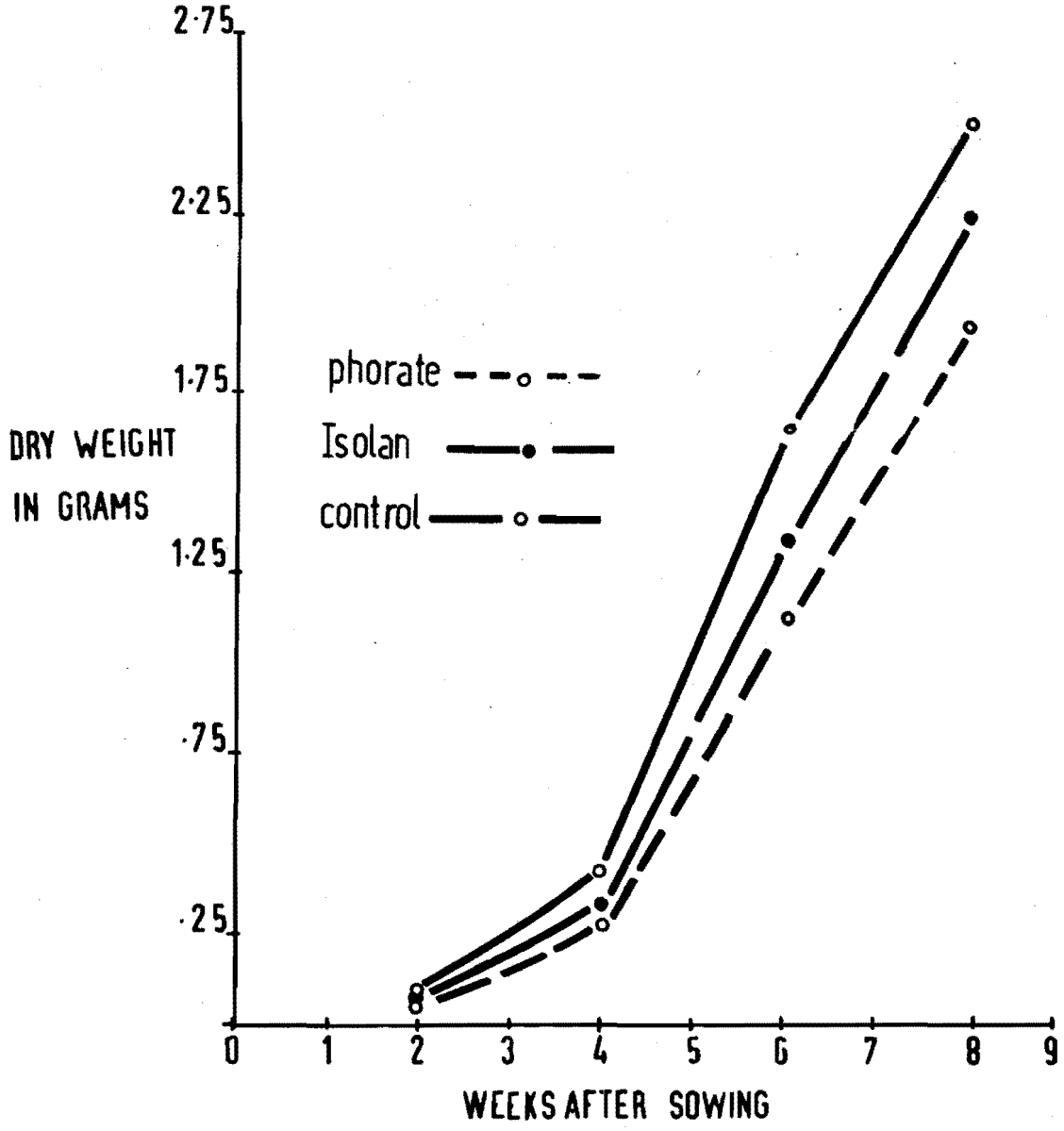
Table VII A Comparison of Dryweights (grams) of Wheat Plants, harvested at 56 days after sowing in soil treated with phorate and Isolan.

Treatments			No. of Replicates				
Insecticide	Placement	Rate lbs.a.i./acre	1	2	3	4	5
Phorate	Infurrow	1 lb.	1.938	1.780	2.383	1.881	2.211
		2 lb.	1.993	2.125	2.092	1.893	1.844
		3 lb.	1.896	1.947	1.670	1.770	1.690
Phorate	Side	1 lb.	2.581	2.363	2.380	2.278	2.077
		2 lb.	2.111	1.749	1.952	2.361	2.172
		3 lb.	1.844	1.447	2.302	2.130	2.148
Isolan	Infurrow	1 lb.	2.463	2.322	2.440	2.337	2.405
		2 lb.	2.138	2.252	2.068	2.310	2.192
		3 lb.	2.193	2.000	1.961	2.404	2.107
Isolan	Side	1 lb.	2.547	2.544	2.469	2.462	2.449
		2 lb.	2.565	2.426	2.371	2.538	2.323
		3 lb.	2.285	2.323	2.266	2.423	2.389
Control			2.269	2.521	2.270	3.231	2.231

FIGURE 4

The stunting effect of phorate and Isolan applied in the seed furrow with wheat.

DRY WEIGHTS OF TREATED AND NON-TREATED PLANTS



Comments resulting from analysis of data (Table VII).

(1) The S.E. for this harvest was (0.203 grams) and the C.V. was found to be 9.51%.

(2) Phorate treatments were more damaging to plant growth than the corresponding treatments of Isolan ($F = 33.4^{**}$).

(3) No interaction between insecticide and placement was evident although infurrow applications of insecticide were more damaging to plant growth than side applications ($F = 12.2^{**}$).

(4) The 3 lb. a.i./acre application of phorate and Isolan both had a more severe effect on plant growth than the corresponding 1 lb. rate ($F = 15.20^{**}$).

(5) Treatment means were compared with the control by using a T test. It was found that Isolan side treatment did not differ significantly from the control. However Isolan infurrow treatment differed from the control at the 5% level while both phorate treatments differed at the 1% level.

Results of infurrow treatments from the four harvests carried out on control and infurrow treatments are shown in graph form in Figure 4.

EXPERIMENT 4. THE EFFECT OF SOIL SURFACE APPLICATIONS OF PHORATE AND ISOLAN ON WHEAT GROWTH.

DESIGN.

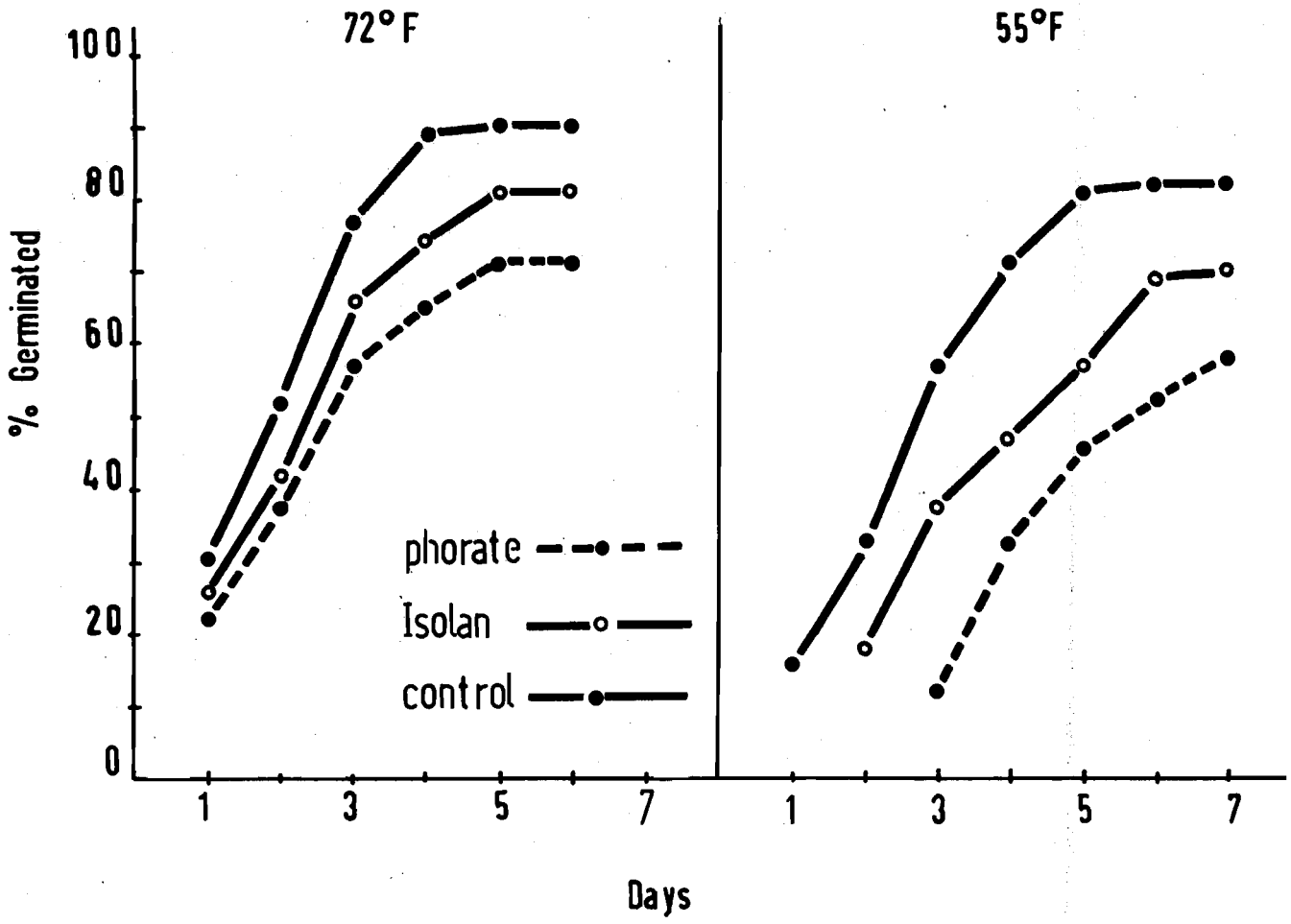
This experiment was initiated to observe the effect of soil surface applications of phorate and Isolan granules on wheat growth. 90 pots were sown with 5 seeds per pot. On emergence the seedlings were reduced to 3 plants per pot. Insecticide was treated to the soil surface of each pot when plants had reached the three true leaf stage. Insecticide treatments administered in this experiment were phorate and Isolan at 2 and 3 lbs. a.i./acre, accompanied with a control.

Fortnightly after treatment, 6 replicates per treatment were harvested. This gave a total of three harvests. From each harvest, dryweights of herbage were recorded.

FIGURE 5

The effect of temperature on the rate and percentage germination of wheat treated with phorate and Isolan.

EFFECT OF TEMPERATURE ON WHEAT SOWN IN TREATED SOIL



RESULTS.

Dryweight recordings showed no significant trends and only readings from the fourth harvest have been recorded, (Appendix XII).

EXPERIMENT 5. EFFECT OF TEMPERATURE ON THE INHIBITION AND RETARDATION OF GERMINATION CAUSED BY PHORATE AND ISOLAN.

DESIGN.

An effort was made to establish more fully the reduction and retardation caused by phorate and Isolan, and to observe the effect these insecticides had on germination of seeds subjected to more adverse conditions.

Selected wheat seeds were sown with an infurrow application of phorate and Isolan at a rate of 2 lbs. a.i./acre, with a non-treated control introduced for each temperature. Temperatures of 13°C and 22°C were chosen for these germination treatments. There were eight replicates in each treatment, each containing five selected seeds. The soil was kept at approximately 50 to 60% field capacity, as outlined under general methods. Additions of water to soil were made daily to treatments subjected to 22°C and every three days to those subjected to 13°C. The results are tabulated in Table VIII.

RESULTS.

Table VIII Percentage of Seed Emergence recorded at daily intervals after the first plant, at 13°C and 22°C had emerged.

Temperature	Treatment	Days						
		1	2	3	4	5	6	7
13°C	Control Not treated	7.5	30.0	57.5	90.0	97.5	97.5	97.5
	Isolan	0	10.0	38.0	47.5	69.8	87.5	87.5
	Phorate	0	0	5.0	30.0	50.0	62.5	72.5
22°C	Control	27.5	62.5	95.0	100.0	100.0	100.0	100.0
	Isolan	31.5	45.0	62.5	92.5	97.5	97.5	97.5
	Phorate	15.0	37.5	57.5	82.5	90.0	90.0	90.0

Comments resulting from data (Table VIII).

It was seen that Isolan treated soil gave no germination at 13°C at the time that germination was observed in non-treated controls. In the case of phorate treated soil, plants did not emerge until two days after the non-treated control. This delay was also shown in results from Experiment 1. These percentages were transformed to $\sqrt{\text{arc sine } \%}$ and graphed, (see Figure 5). Trends from this graph show that when seeds are germinated under adverse conditions suppression and retardation of germination induced by phorate and Isolan becomes more exaggerated.

EXPERIMENT 7. PHYTOTOXICITY OF PHORATE AND ISOLAN IN ASEPTIC CONDITIONS

DESIGN AND METHODS

This experiment was laid down to observe the effect of insecticide on germination, in the absence of most soil pathogens.

Selected seeds were sown under two temperatures on seed germinating pads in petrie dishes. The dishes were moistened with distilled water. The experiment was laid down within a growth cabinet at 13°C and three seeds and three rates of insecticide sprinkled on the germination pads uniformily over a $1\frac{1}{2}$" wide diametral band. This assimilated the area of a furrow. Lids were then replaced. The rates of insecticide were 1 lb a.i., 2 lbs., a.i., and 3 lbs per acre. Each treatment contained six replicates giving a total of eighteen seeds within each treatment. A control was added for each temperature.

RESULTS

Fourteen days after sowing, counts were made and there appeared to be no response to application rates. The percentage of seeds that germinated from plants treated with phorate was 66.7 % while 80 % of the seeds placed on Isolan treated plants germinated. 100 % germination was recorded in control treatments. All seedlings grown on plants treated with both insecticides were severely stunted, phorate treated plants worse than Isolan treated.

The visual results of this experiment are shown on Plate 4 and the typical stunting effects on the plants on Plate 5.

DISCUSSION

Trends from Experiment 1 suggest that phorate as an infurrow application

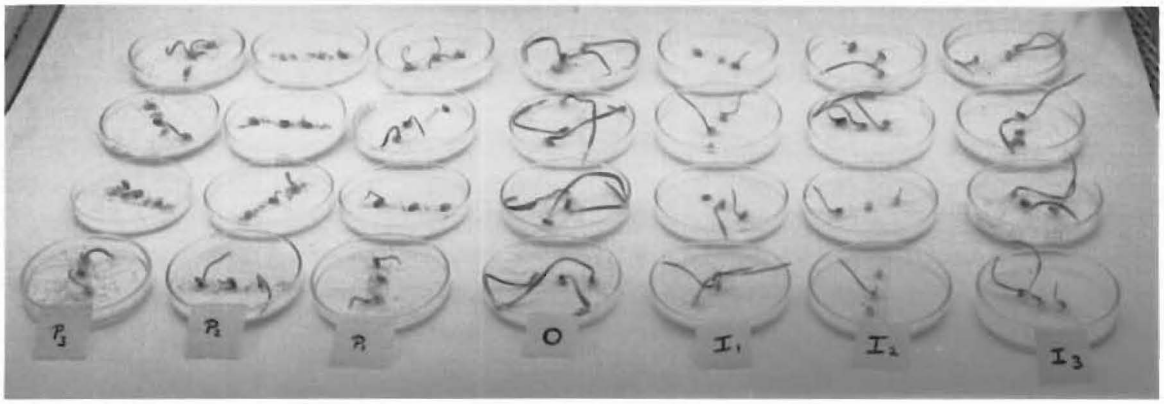
P L A T E 4

Effect of phorate and Isolan on wheat germinated on filter paper.

(0= control I = Isolan P = Phorate 1,2, and 3 denotes lbs a.i./acre)

P L A T E 5

The stunting and retarding effect on the growth of wheat seedlings germinated on filter paper treated with phorate.


P3
P2
P1
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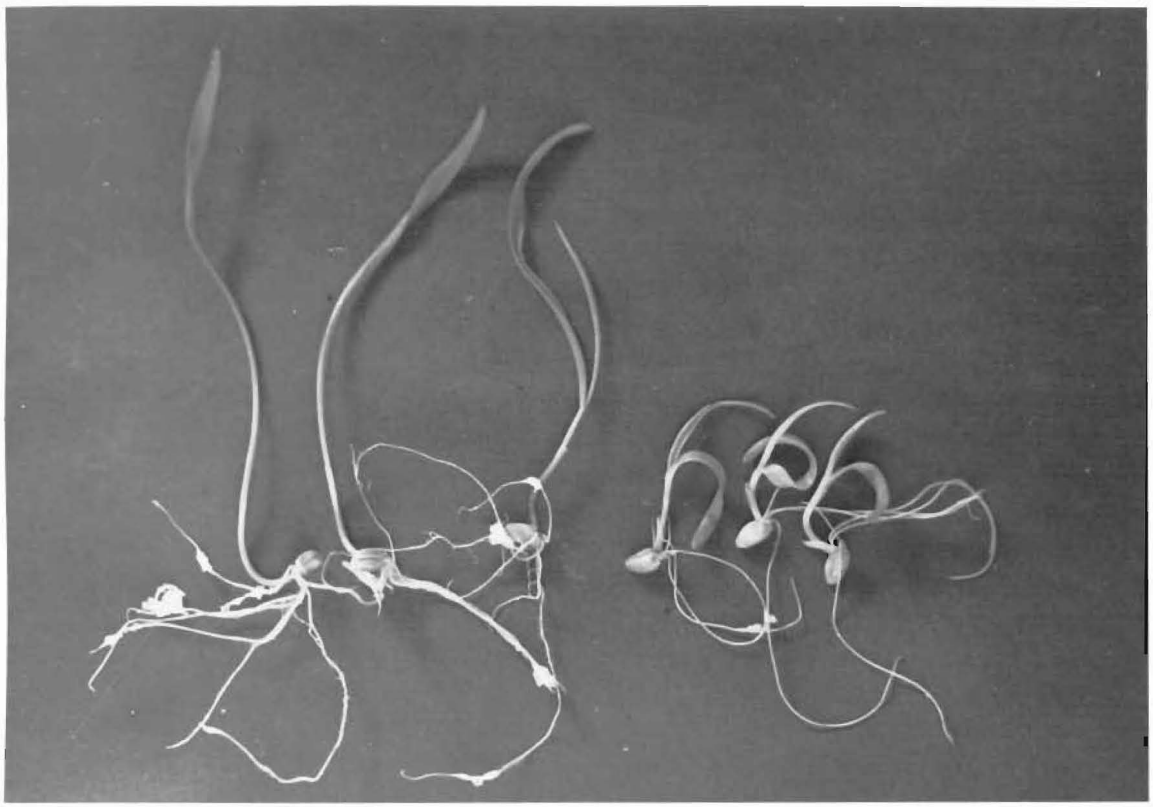
I2
I3



suppresses germination of barley and wheat, whereas oats appear to be more tolerant to this insecticide. Isolan unlike phorate in this experiment, showed no well defined trends in suppressing germination of wheat, oats or barley. The apparent non phytotoxic property of Isolan shown in this experiment is inconsistent with results from further experiments, for which no explanation can be offered.

Infurrow applications of phorate and Isolan retarded germination (Experiment 2). No difference was shown between rates of application although phorate was more severe in retarding emergence than Isolan. Side applications of both insecticides were not significantly different from the control. Emergence was suppressed by both phorate and Isolan as infurrow applications, but no suppression was observed with side applications. Phorate and Isolan did not differ in their capacity to suppress emergence, (in contrast with Experiment 1). The detrimental effect that phorate and Isolan had on the germination of wheat and barley varied in severity from a retardation to suppression.

Observations carried out to note the effect of side and infurrow applications of phorate and Isolan on wheat growth, showed that:-

(a) Side application treatments of Isolan compared with the control were not significantly different over the eight week period.

(b) Isolan infurrow application treatments were inferior to the control in the first harvest, improved and did not differ significantly from the control in the second, but were inferior to the control in the third and fourth harvests. The apparent temporary improvement observed in harvest 2 for this treatment could not be accounted for.

(c) Phorate side application treatments did not differ from the control over the first two harvests but did differ in the third and fourth, due it was thought, to the plant roots coming in contact with side placed deposits of phorate.

(d) Phorate infurrow treatments were vastly inferior to controls over the four harvests.

Phorate treatments were observed to be more phytotoxic at the eight week period than Isolan, and infurrow applications of insecticides were more damaging to plant growth than the corresponding side applications. At the eight week harvest the dry weight of wheat plants compared with the non-treated control was 25.3% greater than the dry weight from the side applications of phorate, 31.66% greater than the plant dry weight from infurrow applications of phorate and 10.3% greater than Isolan infurrow treatments. Phorate side applications as shown by the bioassay (Part I) gave inefficient control of aphids, owing to the restricted movement of phorate through the soil. However this treatment gave a severe stunting of wheat growth at the eight week period. The only feasible explanation for this is that under glasshouse conditions of rapid growth a higher concentration was required within the plant for aphid control than to stunt growth.

No linear rate response was shown in relation to decreasing dry weight over the first six weeks, but this trend was significant at the eight weeks period for both phorate and Isolan treatments. From this it would appear that until eight weeks period all application rates were above an injury threshold level, and a response to rates only became visible as the insecticide was bound and/or metabolised within the soil. This would indicate that wheat plants have the ability to recover as the insecticide content of the soil is depleted.

Damage to the plants induced by these insecticides was a uniform stunting rather than a morphological abnormality. This was concluded from leaf stem, dryweight ratio, leaf number and tiller counts, which were analysed at six weeks period. The six week period was chosen as it offset the effect of slow germination of seed sown in treated soil. No trends were observed in these recordings although marginal scorching of leaves was noticeable with phorate and Isolan infurrow treatments, however this was neither severe or damaging.

Treatments of phorate and Isolan applied to the soil surface of pots in which wheat plants were growing (at the three true leaf stage) gave no deleterious effects on plant growth. Aphid bioassays (Part I) indicated that phorate unlike Isolan when applied to the soil surface was not absorbed by the plant, and was not detrimental to plant growth. Isolan although taken up by the plant did not appear

to effect plant growth.

Stunted wheat growth caused by phorate is not a manifestation of impaired plant vigour at germination, but of a continual uptake by the plant of phytotoxic quantities of the insecticide. This is substantiated by the fact that stunting of wheat plants ensued from applications of phorate sited away from the seed at sowing time. Further evidence was gained from observations of wheat germinated on phorate treated filter paper prior to being sown out in untreated soils. Recovery of these stunted phorate treated seedlings was very rapid. However, from experiments and observations of wheat germination and plant growth it would appear that Isolan impairs the vigour of the germinating plant from which recovery is very slow. This resulted in a stunted wheat plant after eight weeks (flag leaf stage). Side and surface applications of Isolan did not impair plant growth, although bioassays carried out in Part I indicated that Isolan was being absorbed and translocated. In comparison Isolan infurrow applications stunted growth markedly after the eight weeks period. The apparent non-phytotoxicity of Isolan side and surface applications may be due to a diffusion of Isolan through the soil, so that plant roots are in contact with relatively low concentrations of insecticides, which although sufficient to control aphids does not stunt plant growth. In comparison infurrow applications of Isolan are localized near or around the seed and thus expose the plant to much higher concentrations of insecticide. Phorate on the other hand being insoluble in water has a reduced movement through the soil resulting in a high concentration of insecticide at the site of application (where plant roots may penetrate).

Under soil conditions of 60% field capacity the retardation and suppression of emergence in wheat treated with phorate and Isolan as infurrow applications were more severe at 13°C than at 22°C. Under low temperature conditions germination and emergence were slower and conditions such as these leave the seed or sensitive developing embryo exposed to high concentrations of insecticide for a longer period. Bardiner (1960) reported that phorate has the ability to permeate the wheat seed when treated as a seed coat. It is suggested that penetration of wheat seed resulting from infurrow applications of phorate granules may result from contact of the seed with granules and/or absorption in the vapour phase. Isolan on the other hand is miscible in water and is probably absorbed into the seed in solution.

Results from Experiment 7 suggest that both insecticides are toxic to seed germination and this effect may be independent of soil pathogens.

C O N C L U S I O N

From work carried out in the preceding experiments, three properties of granulated soil applied systemic insecticides stand out. These properties are, the efficiency of control, persistence of control, and early protection of emerging plants which have been treated as a seed furrow application at sowing. Such properties observed under field conditions are well documented in the literature.

The above properties give granulated systemic insecticides considerable advantages over the orthodox spray application. These advantages are well suited to control of specific pest problems:

a) control of pests which are present throughout the growing season, or control of pests requiring a persistent insecticide;

b) control of pests which attack crops on emergence, when it is impracticable to spray due to insufficient herbage to constitute an adequate spray target;

c) selective control of phytophagous insects so as to maintain a favourable prey/predator or parasite ratio thus preventing the breakdown of biological control agents.

An example of insect control for which systemic granular formulations are receiving widespread attention is the control of Myzus persicae, the vector of beet yellow virus in sugar beet steckling beds and crops, and potato leaf roll virus in potatoes. The chemical control of this vector and the viruses that it transmits relied for its success upon the early protection of the emerging crop and/or persistent control over the infesting period.

When considering the efficiency and persistence of phorate and Isolan from glasshouse trials for control of R. padi (L.) or other aphids in the field it is imperative to have some idea of the pest's ecology. It is recognised that R. padi (L) in New Zealand has two seasonal flights, autumn and spring. The precise date and duration of these flights, is determined by the weather conditions of that particular season. From observations, wheat sown in late autumn - early winter, may miss the autumn flight of aphids. Autumn sown wheat, not infested in the autumn does not attract aphids of the spring flights. If wheat was sown down with

granules and 4-6 weeks control of aphids resulted from this treatment, then in most years this would enable the cereals to be sown earlier and still pass into the winter uninfested. A treatment such as this would eliminate the risk of late sowing which results in the inability to sow the seed due to excess soil moisture which increases with the onset of winter.

It would appear from these glasshouse trials and from field work reviewed in the literature that phorate granules sown with wheat at rates varying from 1-3 lbs. a.i./acre will render plants toxic to aphids for at least 1-2 months. The persistence of Isolan observed in the glasshouse trials may not be an accurate assessment of the persistence that can be expected in the field, and its water solubility property may result in it diffusing or leaching out of the absorptive root zone area into the sub soil and becoming unavailable for absorption by the plant root. In pot trials this factor was restricted.

An economic disadvantage of infurrow application of granules is that the farmer is committed to control measures, prior to the knowledge of the pest status of cereal aphid in that particular season. Topdressed applications of these granulated insecticides would overcome this disadvantage.

Phorate has been recorded in the literature to give efficient control of a wide range of aphids following this type of application. No literature has been located on the efficiency of Isolan following similar applications. The success of aerial application may rely on one or more modes of action. These are:

a) The contact toxicity of materials applied. Both insecticides have been shown to be strong contact aphicides.

b) The fume toxicity of the insecticides used. Observations on phorate and Isolan have shown that both materials emit fumes that are extremely toxic to the cereal aphid. This fumigant property of phorate has been shown in the literature to give excellent field control of aphids. From laboratory observations and Isolan's property of high volatility, it is highly probable that it would perform in the field in a similar way to phorate.

c) Stomach toxicity resulting from systemic activity. This systemic activity

may be due to uptake by the roots following the movement of the insecticide into the soil and/or uptake by the leaf following contact with the granule and/or foliar absorption in the vapour phase.

Assuming that Isolan's fumigant effect was insufficient to give field control following topdressing of granules, then its ability to move into the soil with moisture may result in efficient control when applied aerially to the crop. Granulated systemic insecticides applied to the foliage would have similar disadvantages to sprays. Early protection of crops cannot be obtained from the fumigant action of insecticides due to the sparse vegetation associated with the newly emerged crop. Fumigants depend for their efficiency upon dense foliage in providing a sheltered micro-climate to retain toxic fumes emitted from the applied insecticides. It is also inconceivable that excellent control of aphids can result solely from the contact toxicity of the granules. As stated, Isolan applied to the foliage of a young crop, unlike phorate, has the ability to move into the soil with moisture and give systemic control. Other advantages of soil applied granulated insecticides that are lost with foliar application are, the selective control of phytophagous insects and a reduction in the persistence of control. It would appear then that granules applied as a topdressing to crops have very few advantages over spray application of insecticides.

Phorate and Isolan in comparison with malathion are very potent contact aphicides and should give excellent control of aphids as spray formulations in the field. In the case of Isolan it is reputed to give efficient control of aphids as a spray at rates where no systemic action can be detected. However, both these aphicides are highly toxic to mammals and for this reason they are not suitable for widespread use as sprays.

Phorate and Isolan when applied to the seed furrow in contact with the seed had a detrimental effect on the rate of germination and the percentage germination of the seed. Phorate had a pronounced effect in stunting plant growth whether the insecticide was applied to the seed furrow or as a side application. Isolan on the other hand was only detrimental to plant growth when applied as an infurrow treatment.

These results on the phytotoxicity of these insecticides may give an exaggerated idea on the phytotoxic properties of phorate and Isolan under field conditions. This exaggerated effect is thought to be caused by localised applications of insecticide giving a high concentration in the immediate vicinity of the plant. With mechanical application to the seed furrow or to the side of the seed (under field conditions) insecticides would be applied in a wider band and thus in any particular area there would not be such high concentrations. However, results from these experiments point out the necessity for low rates and even distribution of application.

It is recommended from work carried out in this thesis that the ideal placement site of granules in relation to the seed should be a compromise between efficiency of control and the corresponding phytotoxicity from the proposed application site. This would depend on the insect pest, insecticide, and the type of crop grown. Phorate being insoluble in water has a very restricted movement in soil. Hence for efficient control it must be sown as close as possible to the seed. Isolan on the other hand has the ability to permeate the soil so may be best sown slightly away from the seed, from where it will still impart excellent control.

SUMMARY

The major facts which emerge from experiments carried out with phorate and Isolan both as soil applied systemic insecticides and contact aphicides are :-

1. Phorate and Isolan as infurrow applications sown with wheat barley and oats gave efficient and persistent control of R. padi (L.) However when phorate was sown away from the seed, or applied to the soil surface of pots in which wheat was growing, inefficient control was recorded. Isolan which is water soluble was shown to give efficient control under all methods of application. The inefficient control registered with side and surface applications of phorate would appear to be due to the slow movement of phorate through the soil, owing to its insolubility in water. Phorate infurrow applications were shown to be more persistent than similar applications of Isolan. Persistence with Isolan was observed to be greater as an infurrow application in comparison with surface and side applications.

2. Phorate and Isolan as contact aphicides when compared with malathion were shown under laboratory conditions, to be superior to malathion at the LD50 level. At the LD95 level Isolan was 7.5 times as toxic as malathion and phorate 1.9 times as toxic. The lower 95 % confidence limit of malathion and the higher limit of phorate converged at the LD95 level. Both phorate and Isolan were shown to emit fumes which are extremely toxic to R. padi (L.)

3. Both phorate and Isolan as infurrow applications gave various phytotoxic symptoms. These were :-

(a) Marginal burning of leaves. This effect was apparent as a slight scorching on the leaf margins of oats, wheat and barley when subjected to infurrow applications at the time of sowing.

(b) Phorate and Isolan when used as infurrow treatments were shown to have the properties to both suppress and retard emergence in varying degrees. These factors were shown to be aggravated by temperatures that are more adverse to rapid emergence but appeared to be of no consequence when

optimal for rapid emergence. Germination on moist filter paper was also suppressed. This would suggest that these materials are toxic to germination and that germination suppression by phorate and Isolan in the soil is not merely a predisposition of the seed to soil pathogen attack.

(c) Observations on the stunting of wheat growth were made over an eight week period. Results showed that phorate whether applied as an infurrow or side application, stunted growth. The differences between the two placements was that the latter delayed stunting until the root came in contact with the insecticide deposit (4-6 weeks after sowing), whereas infurrow applications stunted wheat growth from emergence. Side placement of Isolan in contrast with infurrow applications of Isolan did not stunt growth. Phorate was more severe in this respect than Isolan. A rate response in relation to a decrease in dry weights was only observed for phorate and Isolan infurrow application at the eight week period.

When extra-polating results from glasshouse trials care should be taken as properties of materials that are being screened may appear under these artificial conditions but may not appear under field conditions.

There is little doubt that these insecticides as formulated as granules and applied as infurrow applications will give efficient field control of aphids. Care should be taken with rates of applications to see if phytotoxic symptoms evident in the glasshouse arise in the field.

The role that this type of formulation could adequately play in the insecticide field is to give early and persistent control of virus vectors in wheat, potatoes and sugar beet.

Although both materials are extremely potent contact aphicides they are not suitable for widespread use as sprays as they are extremely toxic to mammals.

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A P P E N D I C E S

Appendix I. Analysis of variance on data obtained on the germination of Barley in Table I, Experiment 1, Part III. Percentages shown in Table I were transformed to arc sine % before analysis.

Treatments on Barley	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F.Ratio
Treated v Control	201	1	201	
Phorate v Isolan	743	1	743	19.06*
Rate 1 v 3 lbs. a.i./acre	81	1	81	
Rate 1+3 v 2 lbs. a.i./acre	.05	1	.05	-
Error	77.95	2	38.97	-
Total Treatment Sum of Squares	1103	6	-	-

Appendix II. Analysis of variance on data recorded in Table II, Experiment 2, Part III. Percentages for the 1-2 day period were transformed to arc sine % before analysis.

Treatments	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F. Ratio
Control v Treated	347.41	1	347.41	16.9**
Top v Side Application	2712.02	1	2712.02	132.6**
Phorate v Isolan	255.76	1	255.76	12.46*
Rate 1 v 3 lbs.a.i./acre	50.00	1	50.00	
Rate 1+3 v 2 lbs. a.i./acre	.06	1	.06	
Interaction between Insecticide and Placement	220.16	1	220.16	10.72*
Error	123.13	6	20.52	
Total Treatment Sum of Squares	3658.54	12		

S.E. = 4.53 C.V. = 9.34%

Appendix III. Analysis of data recorded from Experiment 2, Part III. Data shown in Table III was transformed to arc sine % and each treatment summed before analysis.

Treatments	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F.Ratio
Control v Treated	2448	1	2448	
Top v Side Application	34992	1	34992	19.7**
Phorate v Isolan	2351	1	2351	
Rate 1 v 3 lbs. a.i./acre	9522	1	9522	
Rate 1+3 v 2 lbs.a.i./acre	6	1	6	
Interaction between Insecticide and Placement	3034	1	3034	
Error	10626	6	1771	
Total Treatment Sum of Squares	62979	12		

C.V. = 5.1% S.E. = 42.1

Appendix IV. Analysis of variance on data obtained from plants harvested (1st) 14 days after sowing (Experiment 3, Part III).

Treatments	Sum of Squares	D.F.	Mean Sum of Squares	F. Ratio
Control v Treated	855	1	855	11.8**
Infurrow v Side Applications	3666	1	3666	50.77**
Phorate v Isolan	510	1	510	7.08**
Rate 1 v 3 lbs. a.i./acre	281	1	281	
Rate 2 v 1+3 lbs.a.i./acre	124	1	124	
Interaction between Insecticide and Placement	889	1	889	12.3**
Other Interactions	520	6	86.6	
Error	3755	52	72.2	
Total Sum of Squares	10600	64		

S.E. = 8.5 C.V. = 14.96%

Appendix V. Analysis of variance of data recorded from plants harvested (2nd) 28 days after sowing (Experiment 3, Part III).

Treatments	Sum of Squares	D.F.	Mean Sum of Squares	F. Ratio
Control v Treated	8402	1	8402	
Infurrow v Side Applications	29393	1	29393	10.19**
Phorate v Isolan	24563	1	24563	8.52**
Rate 1 v 3 lbs. a.i./acre	11130	1	11130	
Rate 2 v 1+3 lbs.a.i./acre	22113	1	22113	7.67**
Interaction between Insecticide and Placement	45266	1	45266	15.7**
Other Interactions	4837	6	806	
Error	149874	52	2882	
Total	295578	64		

S.E. = 53.68 C.V. = 14.77%

Appendix VI. Analysis of variance of data recorded from plants harvested (3rd) 42 days after sowing (Experiment 3, Part III).

Treatments	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F. Ratio
Control v Treated	5707	1	5707	12.9**
Infurrow v Side Applications	1540	1	1540	
Phorate v Isolan	8736	1	8736	19.7**
1 v 3 lbs. a.i./acre	0	1	0	
1+3 v 2 lbs. a.i./acre	60	1	60	
Interactions	0	1	0	
Other Interactions	3135	6	522	
Error	22929	52	441	
Total Sum of Squares	42167	64	15.7%	

S.E. = 21.00 C.V. = 15.7%

Appendix VII. Analysis of variance of data recorded from plants harvested (4th) 56 days after sowing, (Experiment 3, Part III).

Treatments	Sum of Squares	D.F.	Mean Sum of Squares	F.Ratio
Control v Treated	4721	1	472	11.5**
Top v Side Applications	4986	1	4986	11.3**
Phorate v Isolan	13710	1	13710	33.4**
Rate 1 v 3 lbs. a.i./acre	6300	1	6300	15.0**
Rate 1+3 v 2 lbs.a.i./acre	40	1	40	
Interaction between Insecticide v Placement		1	4	
Other Interactions	1147	6	191	
Error	21808	52	440.9	
Total Sum of Squares	52716	64		

S.E. = 21.00 C.V. = 9.51%

Appendix VIII. Leaf to stem dryweight ratios taken from Experiment 3, harvest 3, Part III.

Treatment lbs.a.i./ acre	Phorate infurrow					Phorate side					Isolan infurrow					Isolan side				
1	.670	.723	.670	.618	.661	.692	.945	.615	.693	1.190	.655	.572	.633	.724	.667	.756	1.084	.632	.609	.759
2	.862	.918	.638	.619	.911	.638	.717	.657	.640	.591	.732	.705	.894	.635	.571	.795	.668	.761	.574	.703
3	.565	.739	.690	1.327	.613	.763	.924	.763	.687	.771	.729	.974	.811	.729	.714	.713	.668	.659	.815	.888
Control	.520	.640	1.014	.706	.909															

Appendix IX. Tiller numbers taken from Harvest 3 (42 days after sowing), Experiment 3, Part III.

Treatment lbs.a.i./ acre	Phorate infurrow					Phorate side					Isolan infurrow					Isolan side				
1	7	6	5	6	6	7	5	6	6	6	7	5	6	5	6	6	6	7	7	6
2	6	7	7	6	5	5	6	6	5	7	5	7	6	6	6	6	5	7	7	8
3	7	4	6	5	4	6	6	7	5	6	5	6	6	7	4	7	5	6	7	6
Control	6	4	6	6	6															

Appendix X. Leaf numbers recorded from Harvest 3 (42 days after sowing), Experiment 3, Part III.

Treatment lbs.a.i./ acre	Phorate infurrow					Phorate side					Isolan infurrow					Isolan side				
1	25	28	28	26	28	34	29	26	28	27	34	25	27	28	27	29	30	32	30	26
2	31	32	25	28	32	26	33	27	30	33	30	30	30	31	29	30	26	33	31	31
3	35	25	28	24	23	32	34	35	26	31	29	31	27	25	30	31	28	27	31	29
Control	29	27	31	27	30															

Appendix XI. Leaf number, tiller number, and stem/leaf dryweight ratio of plant exposed to soil surface application of insecticides, six weeks after sowing and harvested 28 days after treatment, Experiment 4, Part III.

Insecticide	Rate lbs. a.i./acre	Leaf number					Tiller number					Stem/leaf dryweight ratio				
Control	0	31	32	29	35	30	6	6	6	5	7	.839	.947	.880	.979	.701
Phorate	2	30	22	29	29	28	5	6	5	6	6	1.039	.893	.909	.565	.829
Phorate	3	26	34	31	28	30	6	6	6	6	7	.915	.937	.942	.968	.574
Isolan	2	22	21	24	33	29	5	6	6	6	6	.932	1.015	.936	.950	.960
Isolan	3	35	30	32	29	21	7	6	6	5	6	.795	.848	1.074	1.077	.880

Appendix XII. Dryweights of plants subjected to phorate and Isolan applications made to the soil surface six weeks after sowing and harvested 28 days after treatment, Experiment 4, Part III.

Insecticide	Treatment lbs. a.i./ acre	Replicate Numbers					
Control	-	1.766	1.690	1.762	1.620	1.870	1.578
Phorate	2	1.709	1.428	1.246	1.802	1.890	1.851
Phorate	3	1.789	1.788	1.728	1.800	1.401	1.679
Isolan	2	1.806	1.585	1.747	1.525	1.467	1.742
Isolan	3	1.494	1.682	1.731	1.927	1.510	1.821

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